DRUG DISPOSITION IN CHRONIC RENAL FAILURE: STUDIES WITH PARACETAMOL AND FRUSEMIDE.

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AUTHENTICATION

I hereby declare that this thesis was composed by myself and that the work described, other than that acknowledged to have been performed by others, is my own.

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ABSTRACT

Patients with renal failure react inappropriately to many drugs and have an increased incidence of side effects which may be due to changes in drug absorption, distribution, metabolism and excretion. Active or inactive polar drug metabolites which are normally excreted in the urine will accumulate and patients with end stage disease may depend completely on haemodialysis or continuous ambulatory peritoneal dialysis (CAPD) for their elimination. Despite several single dose studies little information exists about the disposition of paracetamol and frusemide during chronic dosing in patients with renal failure including those maintained on dialysis and this has been investigated further in the present study.

Six patients with end stage disease maintained on CAPD were given 1 g of oral paracetamol. Absorption was normal but plasma concentrations of the glucuronide and sulphate conjugates were greatly increased with little change during the observation period. The extraction capacity of the peritoneal membrane was low and the peritoneal clearance was <7 ml.min^{-1} for paracetamol and these conjugates. The disposition of paracetamol was then compared in 6 healthy volunteers and 6 conservatively managed patients with chronic renal failure taking 1 g 3 times a day for 10 days. The daily plasma concentrations of unchanged paracetamol were significantly higher in the patients (3.1 ± 0.6 versus 1.1 ± 0.3 mg.l^{-1}) suggesting enterohepatic recycling with regeneration by hydrolysis of the conjugates and reabsorption of the parent compound. There was marked accumulation of the glucuronide conjugate (87.0 ± 69.0 versus 3.0 ± 0.5 mg.l^{-1} in the volunteers) which was dependent on the severity of the renal failure. The concentrations of the sulphate conjugate did not accumulate as predicted (25.0 ± 19.0 mg.l^{-1}) possibly due to depletion of inorganic sulphate. Patients with end stage renal failure maintained on haemodialysis were also treated with a similar

regime of paracetamol. Neither the glucuronide nor sulphate conjugate reached the predicted mean plasma concentrations of 569 $\pm$150 and 434 $\pm$92 mg.l^{-1} and extraction ratios of paracetamol and its conjugates were less than 50 %.

The diuretic response of frusemide is related to urinary drug concentration with marked interindividual variation. Single doses of oral and intravenous frusemide (40 mg) were given to 8 healthy male volunteers and 11 patients with renal failure maintained on CAPD (80 mg). In the volunteers, the absorption rate was variable and the bioavailability was 53.6 $\pm$21.3 %. Only one half of the intravenous dose and one third of the oral dose was available for pharmacological action as judged by the urinary recovery. Absorption was markedly delayed (128 $\pm$58 versus 90 $\pm$16 min) but more complete (70.1 $\pm$13.1 %) in the patients. The drug was dependent on non-renal clearance for elimination (61.9 $\pm$20.4 ml.min^{-1}) and the peritoneal clearance was negligible. Ten conservatively managed patients with chronic renal failure received 80 to 500 mg frusemide . Despite the markedly reduced renal clearance (11.2 $\pm$19.4 versus 76.5 $\pm$18.0 ml.min^{-1} in the volunteers) no accumulation was observed and absorption was normal. The urinary recovery of frusemide was dependent on residual renal function. The non-renal clearance was also significantly reduced (43.8 $\pm$12.2 versus 61.4 $\pm$11.8 ml.min^{-1} in the volunteers) but the mechanisms are unknown.

Finally, the effects of paracetamol on the actions of frusemide which are thought to depend on prostaglandin synthesis were studied in healthy female volunteers. Paracetamol pretreatment significantly reduced the frusemide-induced increase in renal excretion of vasodilator prostaglandins and the rise in plasma renin activity. There was no significant effect on the natriuresis or diuresis.

Some changes in the disposition of paracetamol and frusemide in patients with chronic renal failure were as predicted. However, findings such as the failure of predicted

accumulation of paracetamol conjugates, the increased plasma concentrations of unchanged paracetamol, the delayed absorption of frusemide (but not paracetamol) in patients on CAPD and the decreased non-renal clearance of frusemide in conservatively managed patients with chronic renal failure were unexpected and not readily explained.

CONTENTS

CHAPTER ONE

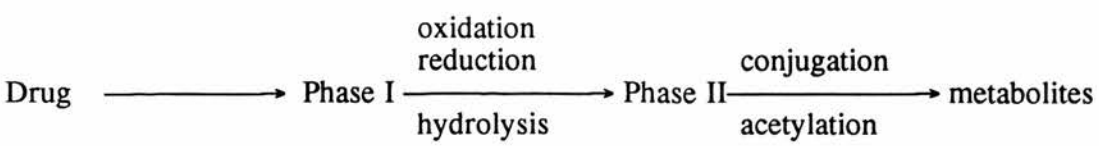
INTRODUCTION AND OBJECTIVES

Patients with impaired renal function often exhibit inappropriate responses to the usual therapeutic doses of drugs and there is a higher incidence than expected of adverse reactions in such patients (Smith et al., 1966 and Jick, 1977). Although the reasons are probably multifactorial, a major determinant is thought to be the alteration in drug disposition which occurs in chronic renal failure (Verbeeck et al., 1981 (a)). This may necessitate a modification of dose (Bennett et al., 1983) particularly when the drug is normally excreted unchanged to a significant extent. Furthermore, pharmacologically active drug metabolites which are removed by urinary excretion will accumulate during regular administration in chronic renal failure leading to a situation analogous to the administration of another unknown drug (Drayer, 1977 and Verbeeck et al., 1981 (a)).

The changes in disposition of drugs which may occur in chronic renal failure include alterations in drug absorption, distribution, metabolism and/ or excretion. For example, gastrointestinal disturbances associated with renal failure such as nausea, vomiting, diarrhoea and oedema of the gastrointestinal tract may affect drug absorption (Lee and Marbury, 1984). Despite this, little information is available regarding the bioavailability of specific drugs in patients with renal failure (Turnheim, 1991). There are reports however of decreased intestinal absorption of frusemide, pindolol and D-xylose in patients with renal failure (Tilstone and Fine, 1978, Chau et al., 1977 and Craig et al., 1983). Patients may be maintained on a variety of medication to treat not only the renal failure but also other conditions. For example, antacids may be prescribed for gastrointestinal symptoms and to lower serum phosphate levels. They may alter the absorption of other drugs by several mechanisms including delayed gastric emptying, simple adsorption of the drug, complex formation between the metal cation and drug and alteration of the gastrointestinal pH (Hurwitz, 1977).

Drug distribution can also be altered significantly in patients with renal disease as a result of reduced binding to plasma proteins (Gibaldi, 1977). For acidic drugs, it is generally accepted that this can be explained by the combination of a decrease in serum albumin concentrations and a decrease in the binding capacity of the albumin due to a change in molecular configuration or to competition for binding by retained anionic metabolites in uraemia (Gibaldi, 1977 and Reidenberg and Drayer, 1984). Drugs that are organic bases may also have decreased binding to plasma proteins in patients with renal disease (Reidenberg, 1977). Drugs which seem to bind normally such as d-tubocurarine and indomethacin may have more than one protein binding site (Ghoeneim et al., 1973 and Sjöholm et al., 1976) whereas others which have decreased binding such as diazepam appear to bind to one only site on albumin (Sjöholm et al., 1976). Decreased plasma protein binding of drugs in patients with chronic renal failure may lead to a higher fraction of unbound drug available for pharmacological action but may also lead to enhanced clearance since the rates of some elimination processes are proportional to the unbound concentration of drug (Gibaldi, 1977).

The majority of drugs are not excreted unchanged but are transformed to metabolites which are then excreted. Renal failure may alter the rate as well as the extent of this biotransformation (Reidenberg, 1977) which often takes place in two phases. In phase I reactions the parent drug is converted to a more polar metabolite by oxidation, reduction or hydrolysis (Drayer, 1974). In phase II, the phase I metabolite or the drug itself reacts with an endogenous substrate such as glucuronic acid, acetyl CoA, glycine or inorganic sulphate to yield a conjugated metabolite which is then excreted in the urine and/or bile (Glauser, 1974).



Most of these reactions are carried out in the liver by microsomal enzymes which are located in the smooth endoplasmic reticulum of the hepatic cells and the more polar metabolites are more easily excreted in the urine than the parent compounds. Drug metabolites may be biologically active or inactive. The former may account for the entire therapeutic activity and toxicity of the parent compound (Drayer, 1977).

The effect of renal failure on hepatic metabolism in animals has been studied by several investigators. A decreased activity of the mixed-function oxidase activity in liver microsomes of uraemic rats was found by Leber and Schütterle (1972), and Patterson and Cohn (1984). However, in man many drugs that are metabolised by microsomal oxidation are eliminated normally in patients with chronic renal failure (Reidenberg, 1977). Indeed, the microsomal oxidation of some drugs is accelerated in uraemia and these include phenytoin (Letteri et al., 1971 and Odar-Cederlöf and Borgå, 1974), antipyrine (Lichter et al., 1973 and Maddocks et al., 1975) and propranolol (Bianchetti et al., 1976). Although decreased plasma protein binding may explain the increased clearance of phenytoin in uraemia, antipyrine is only slightly bound under normal conditions and the binding of propranolol is unaffected by renal disease (Reidenberg, 1977). Induction of oxidation by substances in the diet or other drugs is possible (Reidenberg, 1977 and Loub et al., 1975).

Both reduction of cortisol and hydrolysis of procaine appear to be slower in patients with chronic renal failure (Englert et al., 1958 and Reidenberg et al., 1972) while conjugation of paracetamol with glucuronide and sulphate is reported to be normal (Lowenthal et al., 1976). Acetylation of p-aminosalicylate may be reduced in patients with impaired renal function (Ogg et al., 1968).

Changes in renal drug metabolism must also be considered as the role of the kidneys in drug metabolism is increasingly recognised (Anders, 1980 and Turnheim, 1991).

For example, the osteodystrophy of chronic renal disease is probably a result of impaired 1-hydroxylation of 25-hydroxycholecalciferol in the kidneys (Fraser and Kodicek, 1970). Furthermore, the increased response of patients with renal insufficiency to insulin is not only a result of reduced extrarenal insulin metabolism but also of diminished renal metabolism of insulin (Rabkin et al., 1984).

Reduced renal function will lead to a reduction in the rate of excretion of many drugs via the kidneys. Furthermore, marked accumulation of active or inactive metabolites would be expected to occur under these circumstances (Verbeeck et al., 1981 (a)). For example, allopurinol is rapidly metabolised to oxipurinol which has xanthine oxidase inhibitory activity and significantly contributes to the therapeutic effect of allopurinol in man. Patients with renal failure exhibit a higher incidence of side effects during allopurinol therapy and it has been suggested that this may be due to accumulation of the active metabolite (Elion et al., 1968). The hypothesis has been supported by the finding of greatly elevated plasma concentrations of oxipurinol in patients with renal failure (Hande and Stone, 1979).

Marked accumulation of normally inactive metabolites should also occur with regular administration in patients with chronic renal failure. The consequences of such accumulation are unknown (Prescott et al., 1989). Of particular importance in this regard is glucuronidation which produces a highly polar metabolite which is excreted in the urine and/or bile (Verbeeck et al., 1981 (a)). In renal failure the biliary route of excretion may become more important and under these circumstances the possibility exists for hydrolysis of the conjugate by the gastrointestinal flora with liberation of the parent compound which can be then reabsorbed (Verbeeck et al., 1981 (a)). Oxazepam is eliminated in man almost entirely by formation of the glucuronide conjugate which is normally eliminated in the urine (Alván and Odar-Cederlöf, 1978). Enterohepatic recycling has been proposed to explain the prolonged elimination half life of

oxazepam which occurs during multiple dosing in patients with renal failure and in support of this the faecal excretion of the glucuronide conjugate was markedly increased in such patients (Odar-Cederlöf et al., 1977).

The disposition of drugs is further altered in patients with end-stage renal failure maintained on haemodialysis or continuous ambulatory peritoneal dialysis (CAPD). Dialysis essentially involves the juxtaposition of a semipermeable membrane between a flowing stream of blood and an appropriate rinsing solution (Maher, 1977). In haemodialysis the semipermeable membrane is located in a dialysis machine and in CAPD the patient's own peritoneal membrane is used. Waste products from the patient's blood diffuse across the membrane and are eliminated in the dialysate (Gabriel, 1988). Drugs and drug metabolites can also be eliminated in this way (Lee and Marbury, 1984 and Paton et al., 1985).

The extent of removal of a drug during haemodialysis may be sufficient to require a dosage supplement to ensure adequate therapeutic efficacy (Lee and Marbury, 1984). Numerous factors affect drug removal during haemodialysis and these include:

(1) Physiochemical properties of the drug such as the molecular weight and water solubility.
(2) The mechanical properties of the dialysis system such as the surface area, porosity and thickness of the dialyser membrane and the geometry of the membrane supports.
(3) The blood and dialysate flow rates.
(4) Pharmacokinetic factors such as volume of distribution, intrinsic metabolic clearance, protein binding and red blood cell partition (Lee and Marbury, 1984).

When patients undergo dialysis there may be depletion of drugs administered for therapeutic purposes and dosage may have to be increased. Examples include amino-

glycoside and cephalosporin antibiotics (Maher, 1977). If the intrinsic non-renal or metabolic clearance of a drug is large, the contribution of haemodialysis to the total clearance is likely to be much less and the effect on the overall rate of removal will be minimal. Such drugs include the tricyclic antidepressants (Bickel, 1975).

CAPD is a commonly used alternative to haemodialysis in the treatment of end-stage renal failure. Peritonitis is the most frequent complication of the procedure and the need to administer intraperitoneal antibiotics to treat the condition has stimulated a number of pharmacokinetic studies of these drugs (Keller et al., 1990). There have been relatively few studies with other drugs particularly in relation to removal by the peritoneal route (Keller et al., 1990). Factors which influence the peritoneal clearance of drugs are the volume of distribution and the degree to which the drug is bound to plasma proteins. Thus, prerequisites for rapid drug elimination during CAPD include a small volume of distribution, low plasma protein binding and little non renal or residual renal clearance (Paton et al., 1985 and Keller et al., 1985). The low effective flow rate of the peritoneal effluent of approximately 7 $ml.min^{-1}$ is an important limiting factor (Keller et al., 1990).

Therefore drug disposition may be altered in many ways in patients with impaired renal function. Two of the most commonly used medications in patients with chronic renal failure are the analgesic paracetamol and the loop diuretic frusemide. Both drugs are used frequently and often on a regular basis in such patients.

Paracetamol

Paracetamol (acetaminophen, N-acetyl-para-aminophenol, 4-hydroxy-acetanilide, Fig. 1.1) is frequently recommended as a mild, relatively safe analgesic for patients with chronic renal failure. It is available without prescription in many countries and there has been a marked increase in its use in recent years with a corresponding decline in the use of aspirin (Spooner and Harvey, 1976).

The antipyretic properties of the aniline derivatives were investigated following the discovery in the late 19th century that acetanilide lowered the body temperature of patients with fever. Acetanilde and phenacetin were introduced into clinical practice in 1886 and 1887 respectively and paracetamol was first used clinically as an antipyretic/analgesic by Von Mering in 1893. (Smith, 1958). Paracetamol attracted little attention, however, until the discovery that it was the major metabolite of both acetanilide and phenacetin (Brodie and Axelrod, 1948 and 1949, Fig. 1.1) and that it had similar analgesic potency (Flinn and Brodie, 1948). It was erroneously concluded that the analgesic and antipyretic properties of acetanilide and phenacetin were mainly due to their conversion to paracetamol (Fig 1.1).

Paracetamol has a molecular weight of 151.2 and it is a moderately water and lipid soluble weak organic acid with a pKa of 9.5. It is thus largely un-ionised over the physiological pH range (Forrest et al., 1982). Although it has antipyretic and analgesic actions similar to aspirin it is said to have only a weak antiinflammatory action (Beaver, 1965). Although most antipyretic analgesics are known to act by inhibition of prostaglandins synthesis, paracetamol has been considered in the past to exert only a weak inhibitory effect (Brune, 1983). The administration of paracetamol to healthy female volunteers under conditions of controlled sodium intake, however, caused significant reduction in PGE_2 and sodium excretion similar to that induced by indomethacin (Prescott et al., 1990).

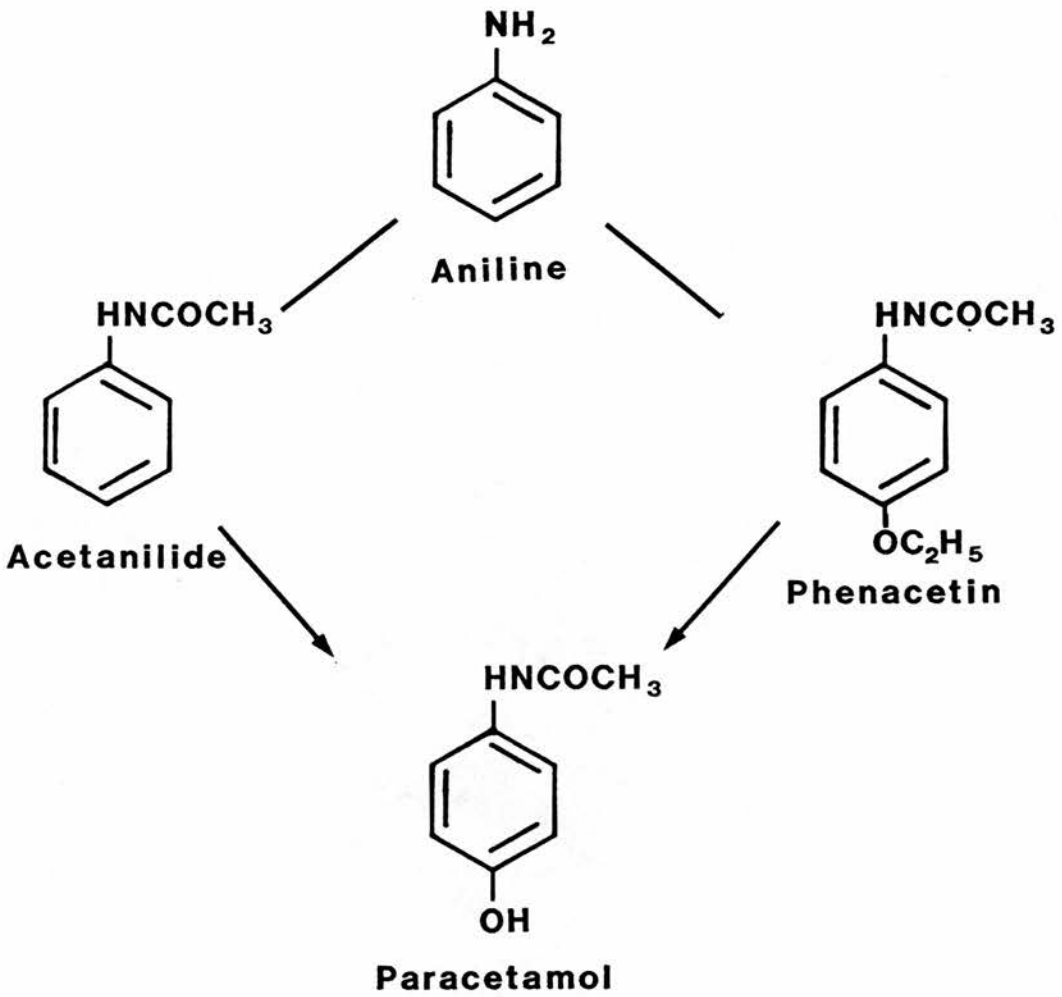


Fig 1.1. Chemical structure of aniline and its derivatives acetanilide, phenacetin and paracetamol.

In healthy volunteers paracetamol is rapidly absorbed from the gastrointestinal tract but a variable proportion is lost through first-pass metabolism (Chiou, 1975, Perucca and Richens, 1979, Rawlins et al., 1977). Oral bioavailability does not increase with larger doses and is about 75 % (Clements et al., 1984). Absorption depends on the rate of gastric emptying (Heading et al., 1973) and animal studies have shown that it occurs rapidly from the small intestine (Bagnall et al., 1979). Mean maximum plasma concentrations in fasting volunteers have been noted at 22 min (Nimmo et al., 1975), 20 min (McGilveray and Mattok, 1972), 21 min (Prescott et al., 1989), 60 min (Dordoni et al., 1973) and 1.4 h (Heading et al., 1973) depending on the dosage form and conditions. There may be as much as an 80 fold range in plasma concentrations 1 h after the ingestion of a therapeutic dose of tablets (Prescott, 1974) and the rate of absorption from solid dosage forms may vary by up to 50 % from one occasion to another (Richter and Smith, 1974).

Paracetamol distributes throughout most tissues and fluids reaching a tissue : plasma concentration ratio of about unity in all tissues except fat and cerebrospinal fluid (Brodie and Axelrod, 1949 and Gwilt et al., 1963). Under normal circumstances paracetamol does not bind extensively to plasma proteins (Forrest et al., 1982) and distribution is largely complete in 1 to 1.5 h. It is extensively metabolised in the liver primarily to a glucuronide and sulphate conjugate. In addition a minor fraction is converted by hepatic mixed function oxidases to a highly reactive alkylating metabolite, probably N-acetyl-p-benzo-quinoneimine (Miner and Kissenger, 1979). This metabolite is normally inactivated by conjugation with reduced glutathione and is eventually excreted in the urine as cysteine and mercapturic acid conjugates (Forrest et al., 1982). Overdoses of paracetamol cause acute hepatic necrosis as a result of depletion of glutathione and of covalent binding of the excess reactive metabolite to vital cell constituents (Mitchell et al., 1973 and 1974).

In young healthy subjects approximately 85 to 95 % of a therapeutic dose is excreted in the urine within 24 h with about 4, 60, 30, 3 and 4 % appearing as unchanged paracetamol and its glucuronide, sulphate, cysteine and mercapturate conjugates respectively (Prescott et al., 1989). As paracetamol is a moderately lipid-soluble weak organic acid it is likely to undergo glomerular filtration with subsequent passive reabsorption and the renal clearance at normal urine flow rates is about 12 $ml.min^{-1}$ (Forrest et al., 1982). The highly polar glucuronide and sulphate conjugates appear to be both filtered at the glomerulus and actively secreted by the tubules since their renal clearances are approximately 130 and 170 $ml.min^{-1}$ (Forrest et al., 1983).

In patients with chronic renal failure, the absorption of paracetamol was normal but from 8 to 24 h it disappeared from the plasma more slowly than in healthy volunteers (Prescott et al., 1989). Furthermore, plasma concentrations of the glucuronide and sulphate conjugate of paracetamol were greatly increased and were even higher in patients with end-stage renal failure maintained on haemodialysis (Lowenthal et al., 1976 and Prescott et al., 1989). Haemodialysis appeared to be the major route of elimination of the metabolites (Øie et al., 1976). Marked accumulation of the polar metabolites would therefore seem inevitable in patients with renal failure taking paracetamol regularly (Prescott et al., 1989).

Paracetamol is normally a very safe drug but may produce acute centrilobular hepatic necrosis when taken in overdose (Clark et al., 1973, Davidson and Eastham, 1966, James et al., 1975, McJunkin et al., 1976 and Portmann et al., 1975). Hepatotoxicity is related to the conversion of a small fraction of the dose to a highly reactive arylating metabolite which is normally inactivated by preferential conjugation with glutathione and excreted as cysteine and mercapturic acid conjugates (Mitchell et al., 1973 and 1974). Following a hepatotoxic dose, glutathione is depleted and the toxic metabolite binds covalently to vital proteins and enzymes causing cell damage and necrosis

(Jollow et al., 1973, Mitchell et al., 1973, Potter et al., 1973 and Prescott 1983). Glutathione precursors and other sulfhydryl compounds prevent glutathione depletion, covalent binding and liver damage, probably by facilitation of glutathione conjugation (Mitchell et al., 1974, Prescott and Matthew, 1974 and Prescott et al., 1977).

Frusemide

Frusemide, (furosemide, (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid or 5-(aminosulfonyl)-4-chloro-2((2-furanylmethyl)amino) benzoic acid, Fig. 1.2) is a potent loop diuretic used in the treatment of oedematous states associated with cardiac, hepatic and renal failure. Therapy is frequently complicated however by apparently erratic systemic availability from the oral route and from unpredictable responses to a given dose (Boles Ponto and Schoenwald, 1990). The extensive literature on frusemide disposition is confusing and frequently conflicting.

The diuretic was first used clinically in 1963 (Kleinfelder, 1963). Early studies confirmed that it was much more effective than the organomercurial agents in inducing a water and sodium diuresis. It could be used orally and its diuretic action was independent of alterations in acid base balance (Goodwin and Gunton, 1965, Stewart and Edwards, 1965 and Verel et al., 1964).

Frusemide is an anthranilic acid derivative with a molecular weight of 330.74. It is practically insoluble in water, only slightly soluble in ether and chloroform, sparingly soluble in alcohol and soluble in acetone, dimethylformamide, acetonitrile, solutions of alkali hydroxides and methanol. The pKa is 3.9. Frusemide is available for both oral and parenteral use.

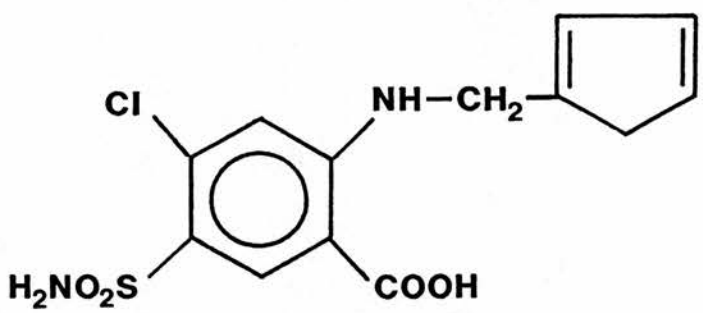


Fig 1.2. Chemical structure of frusemide.

The action of frusemide depends on inhibition of the active reabsorption of chloride at the ascending loop of Henle (Odlind, 1979). At the cellular level, frusemide inhibits the sodium-potassium-chloride cotransport system (Feig, 1986). with enhanced excretion of sodium, chloride, potassium, hydrogen, calcium, magnesium, ammonium, bicarbonate and possibly phosphate. Frusemide is delivered to its site of action by active secretion via the nonspecific organic acid pump (Odlind, 1979 and Odlind and Beermann, 1980 (a)). Response is related to the concentration of the drug in the urine rather than the plasma (Chennevasin et al., 1979) and also to the time course of delivery of drug to the renal tubule (Kaojaren et al., 1982).

The administration of frusemide is associated with a marked increase in the excretion of renal prostaglandins and it has been suggested that they mediate at least some of its pharmacological actions (Abe et al., 1977 and Weber, et al., 1977). In keeping with this the nonsteroidal anti-inflammatory drugs which inhibit prostaglandin synthesis decrease the transient rise in renin activity induced by frusemide (Patak et al., 1975, Rumpf et al., 1975, Fröhlich et al., 1976 and Passmore et al., 1989) but their effects on its natriuretic response are more controversial (Attalah, 1979).

The oral absorption of frusemide is erratic and variable and the reasons proposed include inter- and intra-subject variability, the usage of different dosage forms and study protocols, including whether the subjects were in a fasting or a non fasting state (Boles Ponto and Schoenwald, 1990). The methods used to measure frusemide are also a potential source of variability (Benet, 1979). In general, maximum plasma concentrations of frusemide are achieved 60 to 90 min following an oral dose (Beermann et al., 1975, Branch et al., 1977, Kelly et al., 1974 and Waller et al., 1982) and reported values for the bioavailability range from 20 to 100 % (Branch et al., 1977, Hammarlund et al., 1984, Kelly et al., 1974, Rane et al., 1978, Smith et al., 1980 (a), Tilstone and Fine, 1978, Waller et al., 1982 and Zhu et al., 1987).

Frusemide is highly bound to plasma proteins (>97 %), almost exclusively to albumin (Zini et al., 1976 and Smith et al., 1980 (a)). This together with its limited lipid solubility restricts its distribution throughout the body and the reported values of V_d are 2 to 5 times the plasma volume which is 2.8 to 3.5 l in a 70 kg man (Diem and Letner, 1970).

At least 2 metabolites of frusemide have been reported, frusemide glucuronide and 4-chloro-5-sulfamoylanthranilic acid (CSA). The glucuronide is an accepted metabolite whereas the status of CSA as a metabolite is highly controversial (Smith et al., 1980 (a)). In healthy subjects about 14 % of an oral and intravenous dose of frusemide was recovered in the urine as the glucuronide conjugate (Smith et al., 1980 (a)). The exposure of frusemide to strong acid during the analytical procedure may result in its degradation to CSA (Fig. 1.3) suggesting that the latter is an analytical artifact rather than a metabolite of frusemide (Smith et al., 1980 (a)). The liver may not be the primary site of frusemide metabolism as the non renal clearance of frusemide is not reduced in patients with severe liver disease (Fuller et al., 1981 and Keller et al., 1981).

Following intravenous administration in healthy volunteers frusemide is cleared both by renal and non renal mechanisms in highly variable but often equivalent proportions (Boles Ponto and Schoenwald, 1990). Reported values for total clearance and for the contribution of the renal and non renal components vary enormously (Benet, 1979 and Boles Ponto and Schoenwald, 1990). The fraction of the dose excreted unchanged in the urine represents the amount available for pharmacological action (Brater, 1986). This varies from 50 to 80 % with intravenous administration and from 20 to 55 % with oral administration (Boles Ponto and Schoenwald, 1990).

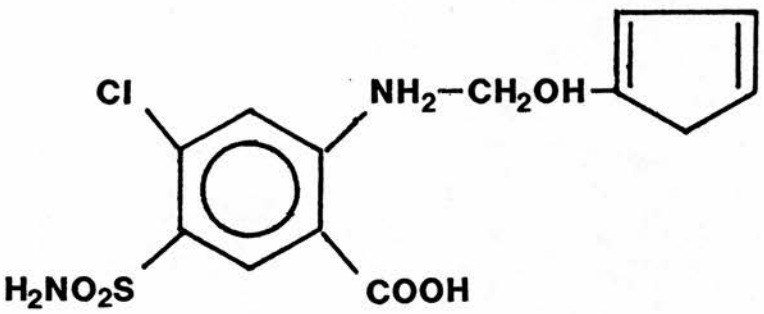


Fig 1.3. Chemical structure of the proposed metabolite of frusemide 4-chloro-5-sulfamoylanthranilic acid (CSA). The status of this metabolite is highly controversial as it may be produced by exposure of frusemide to strong acids during the analytical procedure.

One of the major advantages of frusemide is its ability to induce a diuresis even in patients with advanced renal impairment (Allison and Kennedy, 1971). In patients with chronic renal failure, however, endogenous organic acids accumulate and block frusemide secretion to its site of action at the renal tubules (Rose et al., 1976). Larger doses than normal are therefore required to cause a diuresis (Brater et al., 1986). The disposition of frusemide has been extensively studied in patients with chronic renal impairment but results have been variable and conflicting (Benet, 1979, and Boles Ponto and Schoenwald, 1990). In some studies, the oral absorption of frusemide was delayed in patients with renal failure (Huang et al., 1975, Beermann et al., 1977, Riva et al., 1982 and Boutron et al., 1981). Furthermore the bioavailability was lower than normal (Kelly et al., 1977, Rane et al., 1978 and Tilstone and Fine, 1978).

As expected the renal clearance of frusemide is much lower in patients with renal impairment but the non-renal clearance has been reported to be both decreased (Rane et al., 1978) and increased (Cutler et al., 1974). There has also been controversy over the elimination half life of frusemide which has been reported to vary from normal (1 h) or to as long as 24 h in these patients (Beermann et al., 1977). Although differences may be partly due to differences in study protocols including the assays used to measure frusemide and the nature and degree of renal impairment in the patients studied, nonetheless, at least some of the variability is difficult to explain.

Study objectives

The object of the present study was to elucidate the disposition of paracetamol and frusemide in patients with varying degrees of chronic renal impairment. Both drugs are frequently used in the treatment of patients with chronic renal failure and their disposition in such patients has been investigated following single doses. However, little information is available regarding the situation during multiple dosing in patients with renal failure. As most patients usually take drugs regularly rather than on a once off basis, multiple dose studies are of more relevance clinically.

1. The first objective was to develop a reliable HPLC method for the measurement of frusemide in plasma, urine and dialysate. Paracetamol and its polar conjugates can be reliably measured in plasma using high performance liquid chromatography but frusemide is technically more difficult to measure partly due to its extensive binding to plasma proteins. Inaccuracies may occur due to its sensitivity to light and its proposed instability in the presence of acid which may be required for extraction. Furthermore, it is unclear whether CSA is an analytical artifact or a true metabolite.

2. The disposition of paracetamol in healthy volunteers has been well established but considerable controversy exists in relation to frusemide both in health and disease. The next objective, therefore, was to establish the disposition of single doses of frusemide in healthy drug-free volunteers before attempting to elucidate its disposition in patients with impaired renal function.

3. Little information is available about the disposition of drugs in patients with end-stage renal failure maintained on CAPD. Single doses studies with paracetamol and frusemide were therefore performed in such patients to establish the absorption pattern of both drugs. It was anticipated that abnormalities might exist partly due to the severe renal failure but possibly also due to the presence of large volumes of dialysate

in the peritoneal cavity. It was hoped to establish the effect of severe renal disease on the metabolism of paracetamol to its polar conjugates and the degree to which these metabolites accumulate in the presence of end-stage renal disease. Considerable controversy exists regarding the extent and site of the non-renal clearance of frusemide. It was hoped to clarify the situation in these patients who provide a useful model for non-renal clearance due to the minimal or absent contribution of the kidneys to total clearance. Finally, the peritoneal clearance of frusemide, paracetamol and paracetamol conjugates was measured to find out to what extent this form of dialysis can compensate for the minimal or absent renal clearance.

4. Little information is available regarding drug disposition during chronic dosing. Conservatively managed patients often require large doses of frusemide to maintain an effective diuresis and may be maintained on these doses chronically. A group of such patients was studied to investigate whether the diuretic accumulates under these circumstances, and to determine the absorption patterns of large doses of frusemide. A crucial question was to establish the fraction of the daily doses which reaches its site of action at the tubular lumen. Complementary intravenous studies were performed to establish bioavailability, distribution and the extent of total and non-renal clearance.

5. The next objective was to determine the disposition of paracetamol during chronic dosing in a similar group of patients. It was anticipated that marked accumulation of the polar glucuronide and sulphate conjugates of paracetamol would occur due to reduced renal elimination. In addition, biliary excretion of retained conjugates might lead to regeneration of paracetamol in the gut with subsequent reabsorption of the parent compound leading to persistent and higher concentrations in the blood. It was hoped to determine whether increased production of the potentially toxic glutathione-derived conjugates occurs during chronic dosing in patients with renal failure.

6. A further objective was to establish the disposition of paracetamol in haemodialysis patients under similar conditions to determine the degree of accumulation of the polar paracetamol conjugates in the presence of minimal renal function. It was expected that such patients would be dependent on haemodialysis to eliminate the retained conjugates and it was hoped to establish the adequacy of the procedure in preventing the gross retention of metabolites that would otherwise seem inevitable.

7. The final objective was to investigate the possibility that the pharmacological actions of frusemide including the rise in plasma renin activity, increased excretion of urinary prostaglandins and natriuresis may be inhibited by concurrent treatment with paracetamol due to inhibition of prostaglandin synthesis. Such an interaction might mean that paracetamol is less suitable in patients with renal impairment than was thought, particularly those requiring diuretics.

CHAPTER TWO

METHODS

SECTION 2.1: VOLUNTEERS AND PATIENTS

Healthy volunteers

Healthy male and female volunteers between the ages of 18 to 55 yr were recruited by advertisement or from the hospital staff and their acquaintances. They had no medical illnesses and physical examination was normal. The volunteers were taking no regular medication (apart from oral contraceptives in the case of the females) and they claimed to drink less than 5 units of alcohol per week.

A 10 ml sample of venous blood was drawn from each volunteer for biochemical and haematological screening and urinalysis was performed.

Patients with chronic renal failure

All patients with chronic renal failure who participated in the study were attending the Medical Renal Unit, The Royal Infirmary, Edinburgh. Patients with moderate degrees of renal impairment (i.e. creatinine clearance 10-60 $ml.min^{-1}$) were managed conservatively and those with end-stage disease (creatinine clearance <5-10 $ml.min^{-1}$) were on haemodialysis or continuous ambulatory peritoneal dialysis (CAPD).

The patients were between the ages of 20 to 75 yr and the aetiology of the renal failure included pyelonephritis, glomerulonephritis, polycystic kidney disease, diabetes mellitus and hypertension. Details of the medical histories, medication and the findings on physical examination were noted. Venous blood was sampled to determine current biochemical and haematological status and urine was collected for 24 h to measure the creatinine clearances in the conservatively managed group.

Patients who were treated conservatively for more severe degrees of renal failure were on a low protein diet of about 40 g per day. They were not allowed any added

salt and they avoided foods containing large quantities of potassium. They were encouraged to maintain a high fluid intake. Patients on haemodialysis or CAPD were allowed to eat more protein (60 g per day) but their fluid intake was restricted to about one l per day.

Renal replacement therapy

Haemodialysis

Haemodialysis is an intermittent treatment which is usually performed for 3-5 h, 2 or 3 times a week. During the procedure, the patient's blood is brought into apposition with a dialysate solution in an artificial kidney machine (Fig. 2.1). The blood is separated from the dialysate by a semi-permeable membrane in the dialyser. This has a very large surface area and low molecular weight constituents of the blood such as urea in high concentration pass across the semi-permeable membrane into the dialysate and are eliminated from the body (Allen, 1990 and Gabriel,1988).

Blood is pumped from the patient at about 150 to 200 $ml.min^{-1}$ into the dialyser and a bubble trap and then returned to the patient. Vascular access is usually by a fistula created under the skin between an artery and a vein in the forearm (Fig. 2.1). The vein increases in size as a result of the increased quantity of blood flowing through it, allowing one wide bore needle to be inserted for blood flow to the machine (the "arterial" line) and another for blood returning (the "venous" line).

The dialysate consists of reversed osmosis treated water mixed with a dialysate concentrate in a ratio of 35:1 to give a buffered solution containing sodium 129, chloride 88.8, calcium 1.75, potassium 1.5, magnesium 0.75 and acetate 46.7 $mmol.l^{-1}$, and glucose 0.2% (w/v). It is heated to 37°C and de-aerated before passing through the dialyser at a rate of about 500 $ml.min^{-1}$.

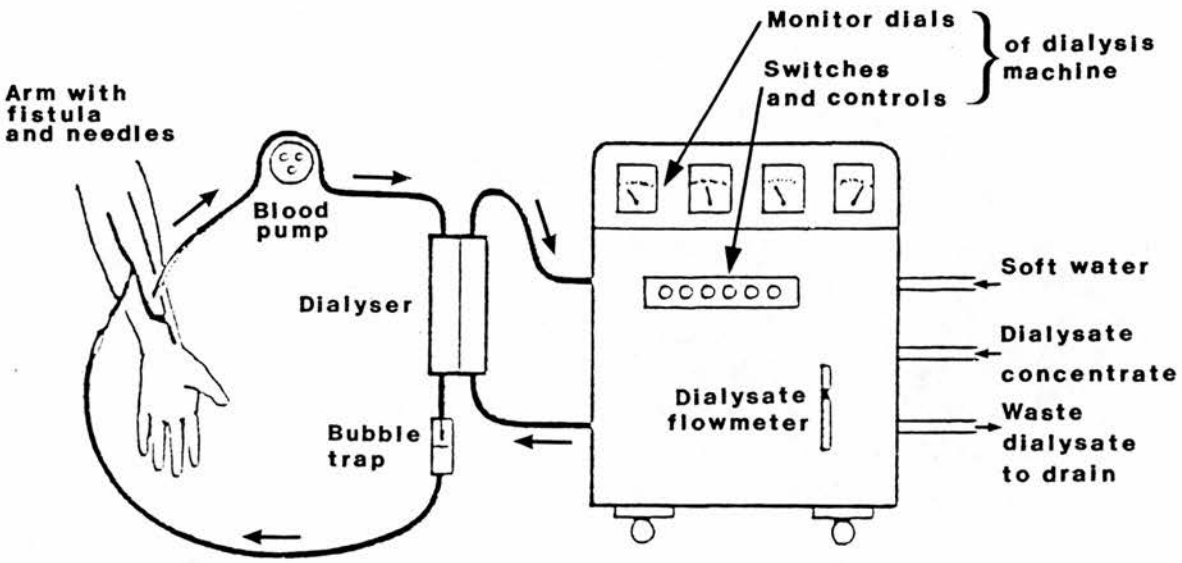


Fig 2.1. A diagram of the basic flow paths of a dialysis machine. The "arterial" blood line leads from the fistula in the arm via the blood pump through the fistula and back to the "venous" line of the fistula. The dialysate pathway extends from the source of dialysate in the machine through the dialyser and then to the drains (adapted from Gabriel, 1980).

The dialyser contains synthetic semi-permeable membranes based on cellulose which allow blood to come into close proximity with the dialysate. If blood and dialysate pass each other at low pressure, low molecular weight metabolites and excess electrolytes pass from the blood to the dialysate and are hence removed from the body. If the pressure across the semi-permeable membrane is raised either by increasing the pressure of the blood in the dialyser or increasing the osmotic effect of the dialysate or both, water will be extracted from the patient. The dialysis machines used were Gambro AK10 (Gambro, Hechingen, Federal Republic of Germany) or Fresenius 2008 (Fresenius, Bad Homburg, Federal Republic of Germany) with hollow fibre dialysers.

Continuous Ambulatory Peritoneal Dialysis

In CAPD, dialysis is performed continuously across the peritoneum which acts as a naturally occurring semi-permeable membrane (Gabriel, 1988). Dialysate is introduced into the abdominal cavity and removed at regular intervals via a flexible "Tenckhoff" catheter which may be left in situ permanently (Fig. 2.2).

The dialysate is similar to that used for haemodialysis except that it is made hypertonic by adding varying quantities of glucose which exert a "sucking" effect on the peritoneum, drawing fluid out from the blood by osmosis. The greater the quantity of glucose, the more fluid is removed. The dialysate comes in sealed bags of 2000 ml and contains sodium 132, chloride 102, calcium 1.75, lactate 35, and magnesium 0.75 $mmol.l^{-1}$ ("Dianeal" solution). The pH is 5.5 and the glucose content is 1.36, 2.27 or 3.86% depending on individual requirements. Some patients on an older system use dialysate containing sodium 134 and chloride 103.5 $mmol.l^{-1}$ with a glucose content of 1.5 or 2.3 %.

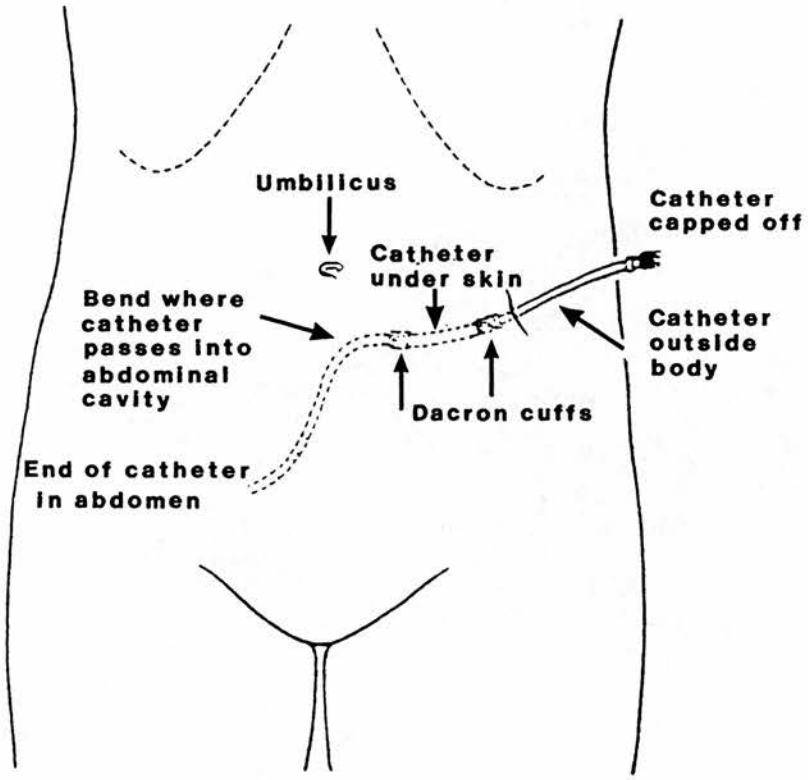


Fig 2.2. A chronic peritoneal "Tenckhoff" dialysis catheter in place (adapted from Gabriel, 1980).

CAPD is performed by connecting a warmed bag of dialysate to the end of the Tenckhoff catheter under sterile conditions . The bag is then hung up above the patient and the dialysate flows into the abdominal cavity (Fig. 2.3). The empty bag is clamped off, rolled up and put under the patient's clothing.

After a dwell time of 5 to 8 h, the first bag is unrolled and placed below the abdominal cavity to drain the used dialysate out of the peritoneal cavity. Once this is complete the bag is disconnected and replaced by a fresh bag which is hung up to allow the dialysate to run in as before.

Most patients perform 3 to 4 exchanges per day at approximately 08.00, 13.00, 18.00 and 23.00 h. The main complication of CAPD is bacterial peritonitis and therefore a strict aseptic technique is essential (Gabriel, 1988).

Ethical approval and subject consent

All studies were approved by the local Ethics of Medical Research Committee and healthy volunteers and patients with chronic renal failure gave their informed written consent before participating in the studies.

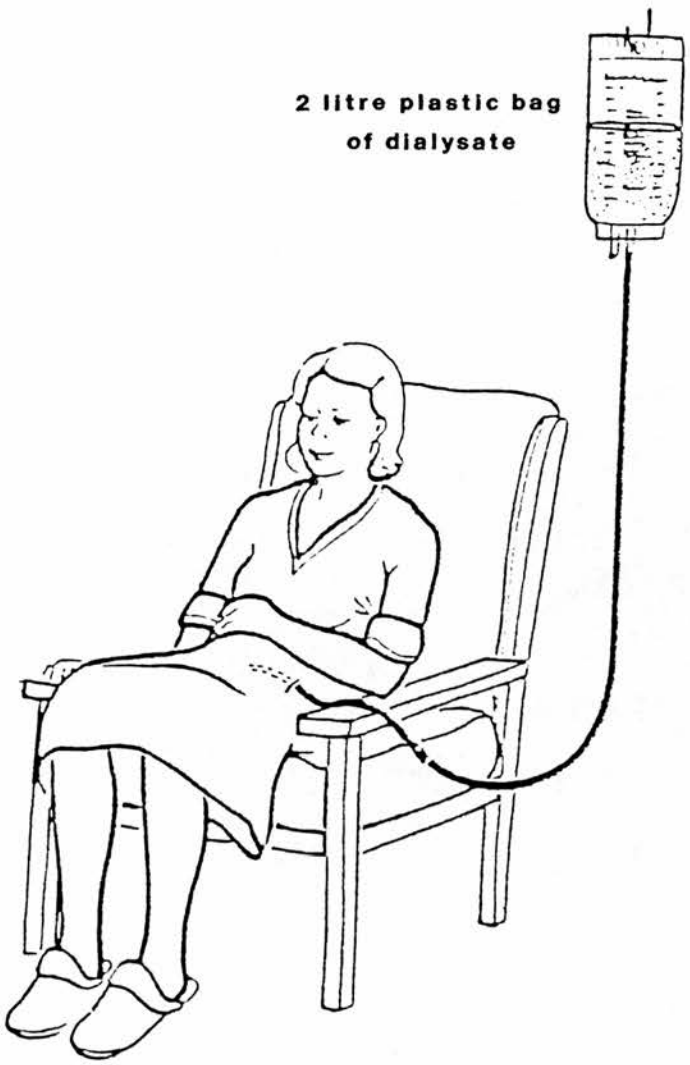


Fig 2.3. The technique of continuous ambulatory peritoneal dialysis (CAPD). A 2 l bag of dialysate is hung up above the patient. The fluid flows down the transfer set into the peritoneal cavity. The line is then clamped off and the bag is rolled up and put into the patients pocket. After 5 h or more the bag is unrolled and placed below the level of the patient's abdomen and the used dialysate flows out (adapted from Gabriel, 1980).

SECTION 2.2: ANALYTICAL METHODS

Concentrations of paracetamol and frusemide were quantitated in plasma, urine and dialysate by High Performance Liquid Chromatography (HPLC). In the case of paracetamol the method had been previously established in our department and an assay was developed for the measurement of frusemide as part of this work.

Assay of paracetamol and its conjugates in plasma, urine and dialysate by High Performance Liquid Chromatography

Background

Samples were assayed for paracetamol and its glucuronide, sulphate, cysteine, and mercapturate conjugates by HPLC with UV detection. The method is based on the original method of Adriaenssens and Prescott, (1978). Electrochemical detection was also used for low concentrations of the cysteine and mercapturic acid conjugates of paracetamol in the presence of potentially interfering peaks (Clements et al., 1984).

Materials

Paracetamol, paracetamol glucuronide, paracetamol sulphate, paracetamol cysteine and paracetamol mercapturic acid conjugates for reference standards were kindly donated by Sterling-Winthrop Research and Development Division, Alnwick, England. Potassium dihydrogen phosphate, formic acid, isopropanol and acetic acid were supplied by BDH Chemicals Limited, Poole, England. 60% perchloric acid and ethyl acetate were supplied by Fisons, Loughborough, England and helium was obtained from the British Oxygen Company.

The internal standard used was N-propionyl-4-aminophenol (NPA). This was synthesised from 4-aminophenol and propionic anhydride (BDH Chemicals Limited) as

described by Adriaenssens (1980).

Instrumentation

The HPLC system consisted of a Waters Associates liquid chromatograph comprising a pump (Model 590) which delivered the mobile phase (degassed with helium prior to use) to an automatic sample injection module (Waters Intelligent Sample Processor 710B, WISP). Before reaching the analytical column the solvent was passed through a 2μ metal filter and a Waters "Guard-PAK" precolumn containing the same packing material as the column.

The analytical column was an 8 mm x 10 cm Waters "Radial-PAK" cartridge containing a reversed phase, 5 or 10μ, C_{18} bonded spherical silica support. The column was housed in a Waters RCM-100 Radial Compression Module. The eluent passed into a Waters Associate ultraviolet (UV) absorbance detector, Model 441. The UV detector output was passed to an IBM-XT microcomputer running the JCL6000 (Jones Chromatography, Clywd, Wales) programme for integration and full analysis of the chromatogram. Results were printed on an Epson LX-800 printer. Electrochemical detection (Model LC-4A, Bioanalytical Systems, Inc.) was also used to detect small amounts of cysteine and mercapturate conjugates as described (Clements et al., 1984).

Collection and storage of samples

Venous blood samples were collected into 10 ml heparinised tubes, centrifuged at 1250 g for 15 min and the plasma transferred to glass tubes. Urinary volumes and pH were recorded and 20 ml aliquots stored in universal containers. For patients on CAPD, used bags of dialysate were weighed and their volumes measured. All samples were stored at -20°C until assayed.

Assay of plasma samples

Chromatographic conditions

Mobile phase:	0.05 M potassium dihydrogen phosphate containing 0.1% formic acid:isopropanol (295:5 v/v)
Flow rate:	3ml.min^{-1}
Pressure:	1000 psi
Detector wavelength:	254 nm

Preparation of standards and test samples for plasma assay

For estimating concentrations of paracetamol and its metabolites, spiked plasma samples containing known concentrations of paracetamol were prepared and run in duplicate with the test samples. To 0.5 ml of plasma was added 50 μl of 30 mg% NPA in 30% w/v aqueous perchloric acid and the sample was then "whirlimixed" (Whirlimixer, Jencons, Hertfordshire, England) to precipitate the plasma proteins. The tubes were centrifuged for 10 min at 1400 g and up to 30 μl of the clear supernatant injected directly into the HPLC system.

Results of plasma assay

Concentrations were calculated in paracetamol equivalents from the peak area ratios of paracetamol and paracetamol conjugates to the internal standard, NPA. The ratios were then multiplied by a factor representing the reciprocal of the regression line for the standards to give the paracetamol concentration in μg.ml^{-1}. Correction factors of 0.835 and 1.027 were used for the glucuronide and sulphate conjugates because only authentic paracetamol standards were used. (Adriaenssens and Prescott, 1978).

If chromatograms of the blank samples contained peaks corresponding to paracetamol or its glucuronide or sulphate conjugates 40 μl of ß-glucuronidase and sulphatase

(Sigma Chemical Co., Poole, England) was added to the sample and the mixture incubted at 37°C overnight. These enzymes liberate free paracetamol from its glucuronide and sulphate conjugate respectively. Therefore, a reduction in the size of these peaks with an associated increase in the size of the paracetamol peak on the chromatogram suggested that the samples already contained some paracetamol conjugates. Allowance was made for this by taking into account the known rate of elimination of paracetamol and its conjugates according to Equation 2.1:

$$C_t = C_0 e^{-K.t}$$

where C_t is the plasma concentration at time "t" and represents the residual amount of paracetamol or its conjugates at each time point when there is measurable amounts in the blank sample.

C_0 is the plasma concentration at time "0" in the blank sample.

K is the elimination rate constant of the drug or its conjugates

and "t" is the time of the sample

Using this equation a set of concentrations were calculated which represented the residual concentrations at each time point. These were then subtracted from the concentrations measured after the dose of paracetamol had been given to give the corrected plasma concentrations of paracetamol or its conjugates.

The retention times of paracetamol glucuronide, paracetamol sulphate, paracetamol and NPA were 2.7, 4.2, 6.3, and 12 min respectively. The system also separated the cysteine (5.3 min) and mercapturate (15.0 min) conjugates of paracetamol with electrochemical detection. The limit of detection of each compound was less than 1 $\mu g.ml^{-1}$. The calibration plots for paracetamol were linear over a range of 5 to 50 $\mu g.ml^{-1}$ and the coefficient of variation for repeated measurements was under 5%

(Adriaenssens, 1980).

Assay of urine samples

The materials and instrumentation were the same as for the plasma assay.

Chromatographic conditions

Mobile phase:	1% acetic acid:ethyl acetate (99:0.5 v/v)
Flow rate:	$3ml.min^{-1}$
Pressure:	1000 psi
Detector wavelength:	254 nm

Preparation of standards and test samples for urine assay

For the estimation of paracetamol and its metabolites in urine, standard solutions of paracetamol were prepared and run with the test samples. The urine was mixed with NPA standard in different proportions according to the concentration of paracetamol and its metabolites present and volumes up to 5 μl were injected directly into the HPLC system.

Results of urine assay

Concentrations of paracetamol and its glucuronide and sulphate metabolites were calculated as described for the plasma assay using correction factors of 1.1 and 1.046 for the sulphate and glucuronide conjugates respectively. No correction factor was used for the cysteine and mercapturate conjugate (Adriaenssens, 1980).

The retention times of paracetamol sulphate, paracetamol glucuronide, paracetamol, NPA, cysteine and mercapturate were 0.9, 2.1, 4.8, 9.7, 11.1 and 12.5 min respectively. The limit of detection of the assay was dependent on the presence of interfering

peaks from endogenous material present in the urine. The calibration plots for paracetamol were linear over a range of 25 to 500 $\mu g.ml^{-1}$ and the coefficient of variation was about 10 % (Adriaenssens, 1980).

Assay of dialysate samples

The same method was used as for the urine samples but because the samples were very dilute larger volumes (10 - 30 μl) were injected into the system.

The measurement of frusemide in plasma, urine and dialysate by High Performance Liquid Chromatography

Background

Many methods have been described for the measurement of frusemide in biological fluids. Early techniques included the administration of the radiolabelled drug, frusemide-S^{35} (Calesnick et al., 1966, Kelly et al., 1974, Beerman et al., 1975 and Honari et al., 1977), and a spectrofluorometric assay which was first developed by Häussler and Hadjú (1964), and subsequently modified by several other groups (Cutler et al., 1974, Kelly et al., 1974, Andreason et al., 1978, Homeida et al., 1977 and Huang et al., 1975). Other assays have been developed with thin layer chromatography (Mikkelson & Andreason, 1977) and gas liquid chromatography (Lindström and Molander, 1974). However, more sensitive methods involve the use of high performance liquid chromatography (HPLC) and many different groups have published either their own methods (Lindström, 1974, Blair et al., 1975, Carr et al., 1978, Nation et al., 1979, Andreason et al., 1981, Kerremans et al., 1982, Uchino et al., 1984, Lovett et al., 1985, Sood et al., 1987 and Lin et al., 1979) or modifications of these original methods (Smith et al., 1980 (a)) & 1981, Rapaka et al., 1982 and Snedden et al., 1982).

There are particular problems associated with the measurement of frusemide in plasma which partly explains why the relevant literature is extensive and at times conflicting. Frusemide is extensively bound to plasma proteins under normal circumstances (>97%) (Zini et al., 1976, Rane et al., 1978 and Andreason et al., 1978), and it has to be separated from them in order to measure its concentration in the plasma. The plasma is commonly acidified with hydrochloric acid (HCL) followed by extraction of the frusemide into an organic solvent such as ether prior to its measurement by HPLC (Lindström, 1974, Carr et al., 1978 and Andreason et al., 1981). The

organic phase is then evaporated and the residue dissolved in a suitable solvent before injection into the HPLC system. However, exposure of frusemide to acid causes its degradation to a split product 4-chloro-5-sulphamoylanthranilic acid (CSA) and complete transformation to CSA is achieved by heating an acid solution of frusemide to 70°C for 45 min (Hadjú & Häussler, 1964). This has lead to the belief by some authors that the use of HCl before extraction is responsible for degradation of frusemide during the analytical procedure with production of CSA as an artifact (Smith et al., 1980 (a))). Kerremans et al., (1982) only recovered 50% of frusemide using 1.5 M HCl and preferred acetic acid for acidification. Other authors have substituted methylene chloride as the solvent for extraction because they found less interference from endogenous substances removed from plasma with the frusemide (Uchino et al., 1984, Lovett et al., 1985).

Another approach is precipitation of plasma proteins with acetonitrile in place of extraction (Lin et al., 1979). The addition of acetonitrile to plasma results in the precipitation of proteinaceous material while the frusemide remains in the organic phase. The acetonitrile is then removed by evaporation and the reconstituted sample injected into the HPLC system, thereby eliminating the need for the acidification of the sample prior to analysis. However, a comparison of both methods showed that with extraction the sensitivity was approximately five times greater than with acetonitrile precipitation (Bauza et al., 1985). Furthermore, even though 6 M HCl was used to acidify the samples to pH less than or equal to 1, the recovery of frusemide was essentially quantitative when extraction followed within 2 min. After 1 h 5-10% of the frusemide was lost, presumably due to decomposition.

The story is further complicated by the methods used for the detection of frusemide in the HPLC system. Several authors have used UV detection at a wavelength 280 nm (Lindström, 1974, Blair et al., 1975, Andreason et al., 1981 and Lin et al., 1979) but

fluorescence detection increases the sensitivity of the assay (Smith et al., 1980 (a))) and it has been adopted by most authors (Carr et al., 1978, Nation et al., 1979, Rapaka et al., 1982, Kerremans et al., 1982, Uchino et al., 1984, Lovett et al., 1985 and Sood et al., 1987). The difficulty arises from the fact that the fluorescence of frusemide is greatest at acid pH but here it is unstable (Kerremans et al., 1982). If the fluorescence of frusemide at pH 1.6 is taken as 100%, it is 97% at pH 2.5 and 81% at pH 4.6. There is no appreciable fluorescence at pH 5.6 or 6.6 (Rapaka et al., 1982). Therefore, even if samples are not exposed to strong acids during extraction, most authors use mobile phases which have been buffered to low pH in the range of 2.0 - 3.5 (Kerremans et al., 1982, Rapaka et al., 1982, Blair et al., 1985 and Lovett et al., 1985). This raises the question of stability of the frusemide under these conditions.

Decomposition of frusemide may also occur when it is exposed to light (Moore and Sithipitaks 1983), and exclusion of light is recommended during storage and analysis (Blair et al., 1975, Carr et al., 1978, Kerremans et al., 1982, Uchino et al., 1984, Sood et al., 1987 and Bauza et al., 1985). When solutions of frusemide were exposed to light, there was rapid hydrolysis to at least 3 compounds and this process was markedly accelerated by an acidic pH (Kerremans et al., 1982). However it has been subsequently been claimed that special treatment to protect the samples from light is unnecessary provided that frusemide is stable in each of the solvents used for the assay upon exposure to normal fluorescent room lighting (Lovett et al., 1985). Frusemide also remains stable when it is stored in frozen plasma for at least 4 months (Kerremans et al., 1982 and Lovett et al., 1985).

The pharmacological activity of frusemide seems to correlate best with its urinary excretion rate (Chennavasin et al., 1979) and most authors have included the estimation of urinary frusemide in their methods. What is puzzling is that these methods often involve elaborate extraction procedures despite the fact that less than 100 mg

protein is normally excreted per day in the urine (Lindström, 1974, Carr et al., 1975, Kerremans et al., 1982, Uchino et al., 1984, Sneddon et al., 1982 and Bauza et al., 1985). By contrast the concentration of proteins in the plasma is normally 60-80 $g.l^{-1}$. It seems unnecessary therefore to remove these small amounts urinary protein under normal circumstances. Interference from endogenous substances may also have necessitated extraction although urine has been injected directly onto the HPLC column (Lin et al., 1979). The same stability problems apply to frusemide in urine on exposure to light or acidic pH but samples can be stored frozen for 204 days (Bauza et al., 1985).

On the basis of these reports precautions should be taken to minimise the exposure of frusemide samples to acid or light during the analytical procedure.

Development of HPLC assays for the estimation of frusemide in plasma, urine and dialysate.

Materials

A pure sample of frusemide was kindly donated by Hoechst UK Limited, Hounslow, Middlesex, England. Acetic acid, acetonitrile, methanol, sodium acetate and the internal standard (toluic acid) were all supplied by BDH Chemicals Limited. Helium and nitrogen were obtained from The British Oxygen Company.

Instrumentation

The HPLC system consisted of a Waters Associates liquid chromatograph comprising a pump which delivered the mobile phase (degassed with helium prior to use) to an automatic sample injection module the Waters Intelligent Sample Processor 710B (WISP). Before reaching the analytical column the solvent passed through a 2μ metal filter and a pre-column containing the same packing material as the column.

The column was a 8 x 100 mm Waters μBondapak C_{18} radial compression cartridge containing ODS silica (10μ). From the column the eluent passed into an Waters Associates ultraviolet (UV) absorbance detector, Model 440. The detector output was passed to a Bryans 28000 single channel recorder (Bryans, Mitcham, England) or a Shimadzu C-R1B reporting integrator (Dyson Instruments, Tyne and Wear, England) to produce the chromatograms and integrated peak areas. Once the assay had been fully standardised the detector output was passed to an IBM-XT microcomputer running the JCL6000 (Jones Chromatography, Clywd, Wales) programme for integration and full analysis of the chromatogram. Results were printed on an Epson LX-800 printer.

Collection and storage of samples

Samples were collected and stored as described for the paracetamol assay but were protected from light at all times.

Chromatographic conditions

Mobile phase:	180 ml sodium acetate (0.01 M)
	70 ml acetonitrile
	30 ml methanol
	200 μl acetic acid
	pH= 5.5
Flow rate:	2 $ml.min^{-1}$
Pressure:	1000 psi
Detector wavelength:	280 nm

Development of plasma assay

(a) Effects of pH on stability of frusemide.

A series of experiments was done with aqueous solutions of frusemide to establish the effects of pH on the stability of frusemide.

A range of aqueous standards of 1, 5 and 10 $mg.l^{-1}$ frusemide were buffered to pH 2 to 7 and volumes of 10 to 30 μl were injected into the system. Frusemide peaks were sharp and well defined from pH 2 until pH 6. As the pH increased beyond 6.3, the peaks of both the frusemide and internal standard became split and obliterated and eventually no frusemide or toluic acid was detected at pH 7.0. Under these conditions no obvious degradation of frusemide occurred under acidic conditions of pH as low as 2.0. The distortion seen at high pH was considered to be an analytical artifact due to interaction with the column and both frusemide and the internal standard disappeared progressively. The manufacturers of the μBondapak column advise using a pH range of 2 to 8 and preferably an even narrower range of 3.5 - 6.5. At a pH of greater than 7.8 the ODS is stripped.

(b) Effects of light on stability of frusemide.

When aqueous samples of frusemide 1, 5 and 10 $mg.l^{-1}$ were exposed to daylight and analysed weekly over a period of 2 months there was a progressive reduction in the size of the peak with development of a second, earlier peak.

(c) Removal of protein from plasma samples.

Plasma proteins were precipitated with acetonitrile as described by Benet et al., (1979). In this way, the need for acidification before extraction was avoided. Initially, 400 μl of acetonitrile was added to 200 μl plasma containing frusemide and internal standard. The sample was centrifuged and 10 to 30 μl of the supernatant was in-

jected directly into the HPLC system. However, the resultant chromatograms showed a number of peaks which did not correspond to frusemide.

(d) Acidification after acetonitrile precipitation.

The chromatograms obtained after acetonitrile precipitation resembled the aqueous standards made up in alkaline buffer. As the pH of plasma is normally 7.35 to 7.45 it seemed reasonable to acidify the samples after acetonitrile precipitation to give good resolution of the peaks. Acidification with 20 μl citric acid (0.01 M) gave clean, sharp reproducible peaks. Unfortunately this resulted in rapid deterioration in column efficiency with broadening of the peaks.

(e) Removal of acetonitrile.

It became apparent that it was acetonitrile itself rather than the pH of the sample that was causing the apparent splitting and distortion of the chromatograms. If acetonitrile was added to aqueous standards, a similar distortion of the shape and size of the frusemide and toluic acid peaks occurred which could be rectified by the addition of citric acid. It was therefore decided to remove the acetonitrile by evaporation with a stream of nitrogen after precipitation and then reconstitute the pellet in de-ionised water before injection into the HPLC system.

Final method for measuring frusemide in plasma by HPLC

Serial dilutions of a stock solution of frusemide in blank plasma (Blood Transfusion Service) were prepared to give 2 sets of standards of concentrations 0.5 to 10 $mg.l^{-1}$ and 0.1 to 0.5 $mg.l^{-1}$. A blank sample of plasma was also included. To 200 μl of each standard was added 20 μl toluic acid (1 $mg.ml^{-1}$) and 400 μl acetonitrile. The sample was whirlimixed and then centrifuged at 1800 g for 10 min. The supernatant was transferred to glass conical tubes and the acetonitrile evaporated off under a gentle stream of nitrogen. The residue was reconstituted with 100 μl of de-ionised water

and 30-90 μl injected into the HPLC system. For the low range of 0.2 to 0.5mg.l^{-1} the mobile phase was changed to use a slightly less acetonitrile (62 ml). Using the peak area ratios of frusemide to internal standard a factor was calculated which represented the reciprocal of the slope of the regression line of the standards. This was then used to calculate the concentration of frusemide in samples from patients and volunteers by multiplying it by the peak area ratios of these unknown samples.

If the blank samples contained peaks with the same retention time as frusemide the values were subtracted from the plasma concentrations to give the true frusemide concentrations. However, when patients were taking frusemide regularly it was assumed that such peaks represented residual amounts of frusemide and this was taken into account by using Equation 2.1 as described for paracetamol.

Results of plasma assay

Chromatograms from drug free plasma and from plasma containing frusemide 4.7 mg.l^{-1} are shown in Fig. 2.4. The retention times of frusemide and the internal standard were 5.4 and 6.7 min respectively. The detection limit of the plasma assay was 0.2mg.l^{-1}.

The high and the low range of plasma standards were assayed on 6 different occasions over a 2 week period and results are shown in Tables 2.1 and 2.2 and Fig. 2.5 and 2.6 respectively. The calibration plots were linear in the range 1 to 10 and 0.2 to 0.5 mg.l^{-1} and the coefficients of variation were 3.3 and 6.9 % respectively.

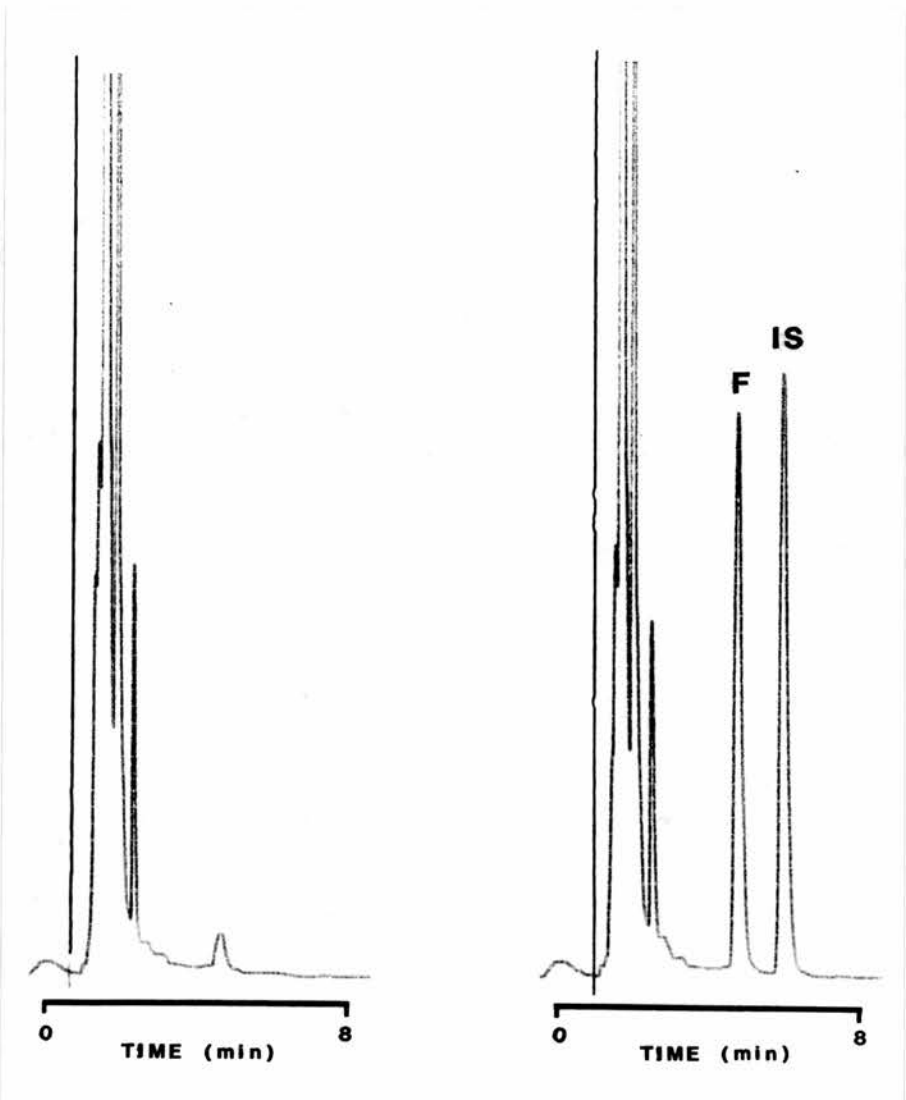


Fig 2.4. Chromatograms obtained from (a) drug free plasma and (b) plasma obtained from a patient with end stage renal failure maintained on CAPD 90 min after an oral dose of 80 mg frusemide. The peaks are frusemide 4.7 $mg.l^{-1}$ and internal standard.

Table 2.1. The precision of the plasma frusemide HPLC assay from 1 to 10 $mg.l^{-1}$ during 6 standard runs with calculation of the coefficient of variation (CV %).

Frusemide concentration ($mg.l^{-1}$)	Peak area ratio Frusemide/Internal standard Run No. 1	2	3	4	5	6	mean ±sd	CV %
1	0.25	0.26	0.28	0.27	0.25	0.26	0.26 ±0.01	4.1
2	0.44	0.45	0.44	0.48	0.44	0.46	0.45 ±0.01	3.2
4	0.84	0.86	0.89	0.84	0.81	0.81	0.84 ±0.03	3.3
6	1.29	1.24	1.21	1.28	1.24	1.19	1.24 ±0.04	2.8
8	1.82	1.74	1.67	1.74	1.73	1.65	1.73 ±0.06	3.2
10	2.11	2.26	2.08	2.19	2.13	2.11	2.15 ±0.06	2.8

Table 2.2. The precision of the plasma frusemide HPLC assay from 0.2 to 0.5 $mg.l^{-1}$ during 6 standard runs with calculation of the coefficient of variation (CV %).

Frusemide concentration ($mg.l^{-1}$)	Peak area ratio Frusemide/Internal standard Run No. 1	2	3	4	5	6	mean ±sd	CV %
0.2	0.45	0.36	0.36	0.31	0.37	0.32	0.36 ±0.05	12.5
0.3	0.63	0.65	0.69	0.65	0.65	0.74	0.67 ±0.04	5.5
0.5	1.06	1.10	1.05	1.00	1.05	1.06	1.05 ±0.03	2.8

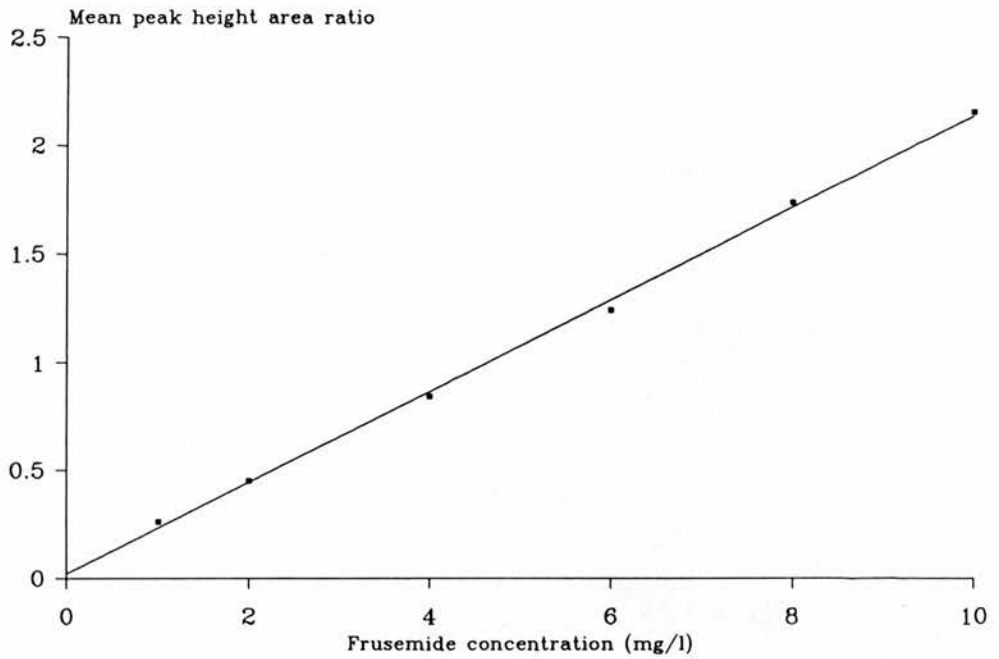


Fig 2.5. Linearity of the HPLC plasma assay for frusemide: Calibration plot of the mean peak area ratio obtained following repeated analysis (n=6) of plasma containing frusemide concentrations in the range 1 to 10 mg.l^{-1}.

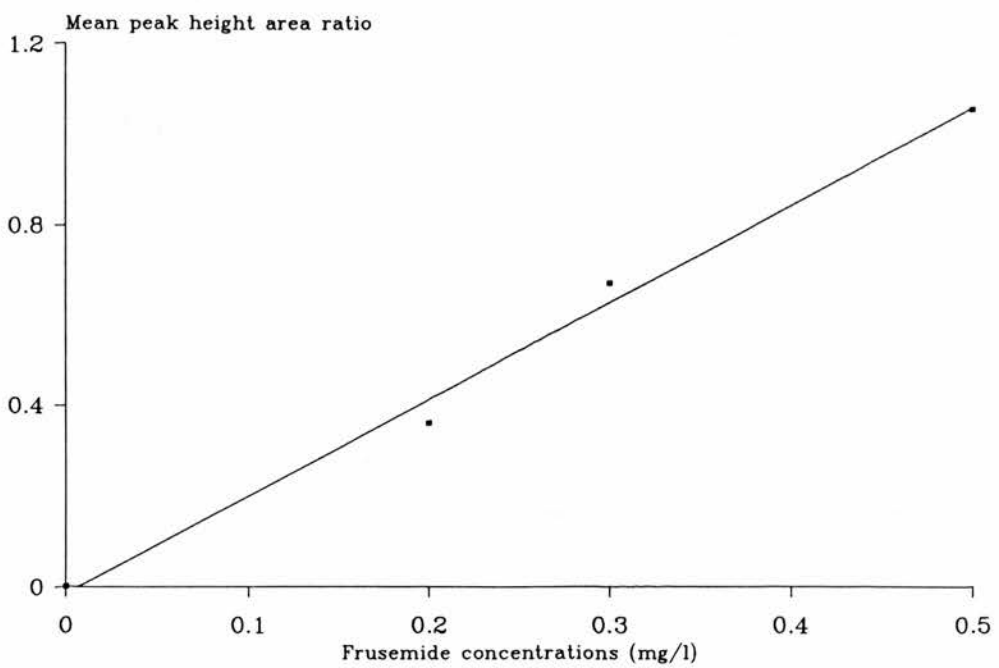


Fig 2.6. Calibration plot of the mean peak height area ratio obtained following repeated analysis (n=6) of plasma containing frusemide 0.2 to 0.5 mg .l^{-1}.

Measurement of frusemide in urine

As there was no interference from endogenous peaks, the urine samples were injected directly into the HPLC system. For estimating the concentration of frusemide in urine, standard solutions were made up with final concentrations ranging from 1 to 10 $mg.l^{-1}$. To 200 μl of each standard sample was added 20 μl of toluic acid (1 $mg.ml^{-1}$). The sample was whirlimixed and injected directly onto the HPLC column. The factor representing the reciprocal of the slope of the regression line for the standards was calculated and this was then multiplied by the peak area ratios of the unknown samples from the patients or volunteers.

Results of urinary assay

Chromatograms from drug free urine and from urine containing frusemide 8.6 $mg.l^{-1}$ are shown in Fig. 2.7. The retention times of frusemide and the internal standard were 5.4 and 6.7 min respectively. The detection limit of the urinary assay was $0.5mg.l^{-1}$.

Urinary standards were assayed on six different occasions over 1 week and results are shown in Table 2.3 and Fig. 1.8. The calibration plot was linear in the range 1 to 10 $mg.l^{-1}$ and the coefficient of variation was 6.8 %.

Measurement of frusemide in dialysate

The same method was used to measure frusemide concentrations in dialysate as was used for urine.

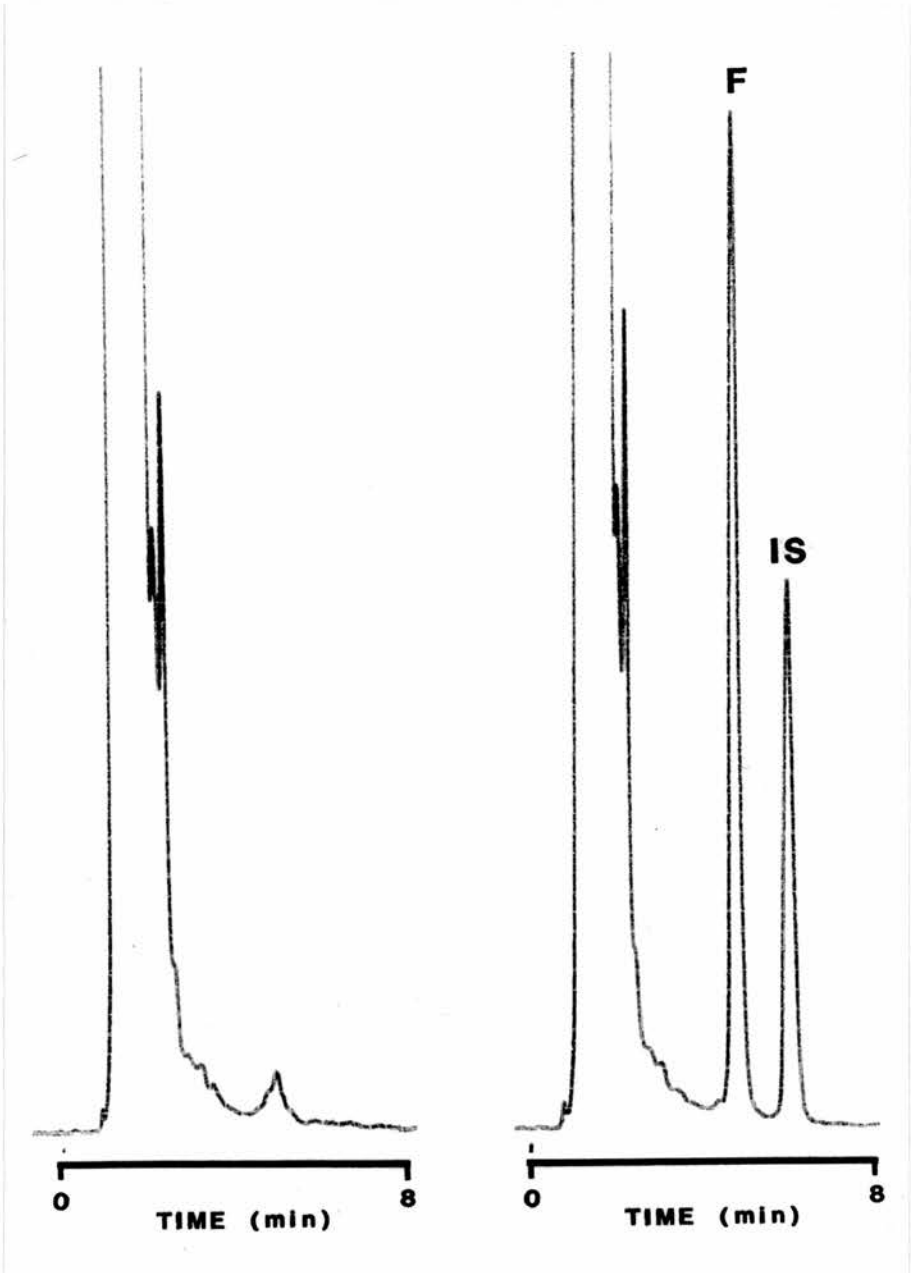


Fig 2.7. Chromatograms obtained from (a) drug free urine and (b) urine collected from a healthy volunteers 2-4 h after the administration of frusemide 40 mg orally. The peaks are frusemide 8.6 $mg.l^{-1}$ and internal standard.

Table 2.3. The precision of the urine fusemide HPLC assay from 1 to 10 $mg.l^{-1}$ during 5 standard runs with calculation of the coefficient of variation (CV %).

Frusemide concentration ($mg.l^{-1}$)	Peak area ratio Frusemide/Internal standard Run No. 1	2	3	4	5	mean ±sd	CV %
1	0.17	0.15	0.14	0.14	0.15	0.15 ±0.01	7.3
2	0.30	0.30	0.28	0.34	0.20	0.30 ±0.02	6.7
4	0.70	0.66	0.69	0.59	0.50	0.63 ±0.07	11.9
6	1.02	0.94	0.84	0.85	0.88	0.91 ±0.07	7.4
8	1.29	1.14	1.14	1.10	1.12	1.16 ±0.07	5.8
10	1.60	1.54	1.57	1.60	1.56	1.57 ±0.02	1.5

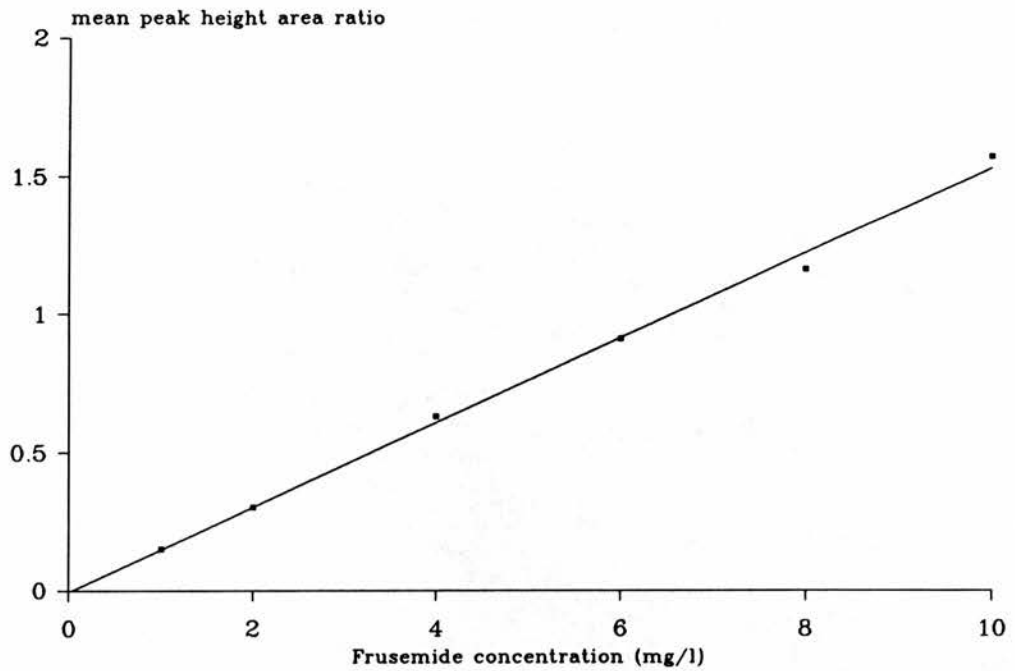


Fig 2.8. Linearity of the HPLC urine assay for frusemide: Calibration plot of the mean peak height ratios obtained following repeated analysis (n=5) of urine containing frusemide concentrations in the range 1 to 10 $mg.l^{-1}$.

Measurement of plasma renin activity (PRA) by radioimmunoassay

PRA activity was measured by radioimmonoassay (RIA) of angiotensin 1 generated under standard conditions (Haber et al., 1969). The intra- and inter-assay coefficients of variation were 4 % and 6 %, respectively.

Measurement of urinary prostaglandin E_2 (PGE_2) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF1_\alpha$) by RIA

PGE_2 was measured in urine using the RIA (kit number NEK020) supplied by Du Pont U.K., Ltd., Stevenage, Hertfordshire, England. The intra- and inter-assay coefficients of variation were 7 % and 14 % respectively. Urinary 6-keto PGF_{1a} was measured by RIA using a modification of a method described by Dray et al., (1975). The intra- and inter-assay coefficients of variation were 7 % and 19 % respectively.

Urinary Sodium

Urinary sodium was measured by an ion selective electrode (Radiometer Ltd., U.K.).

SECTION 2.3 PHARMACOKINETIC AND STATISTICAL ANALYSIS

Presentation of data

Results (mean ±standard deviation, s.d.) were presented both in tabular and diagrammatic form. The data were entered onto an Amstrad PC2086/30 micro computer containing a spread sheet programme ("SuperCalc 5") and the "SIPHAR" pharmacokinetic and statistics programme (SIMED, 1988).

Pharmacokinetic analysis

Pharmacokinetics deals primarily with the processes of drug absorption, distribution and elimination and it is the mathematical description of the time course of changes in the concentration of drugs and their metabolites in the body. Analysis is often based on the use of mathematical models whereby the body is depicted as a system of interconnected compartments each characterised by its volume and the relationships between the rates of input and output of the drug. The simplest case is the one compartment model in which it is assumed that a drug is introduced into the circulation, distributed throughout the body virtually instantaneously and subsequently eliminated at a rate proportional to its concentration (Gibaldi and Perrier, 1982).

In practice the tissue uptake and distribution of drugs takes time and a 2 compartment model is often more appropriate. The body is considered as 2 separate but interconnected compartments, i.e. a central compartment consisting of the circulation and well perfused organs into which the drug distributes quickly, and a peripheral poorly perfused compartment representing the rest of the body where uptake is slower (Gibaldi and Perrier, 1982). In such cases 2 phases are seen in the plot of log concentration versus time following a rapid intravenous injection (Fig. 2.9). This curve is the result of 2 simultaneous exponential processes: a rapid distribution and a slower elimination phase.

Method of residuals

The method of residuals can be used to resolve the drug plasma concentration-time curve shown in Fig. 2.9 into the 2 exponential components. The terms "curve stripping" or "peeling" are used to describe this technique. The first steeper part of the curve represents mainly distribution of the drug. The latter part is referred to as the beta (ß) phase and the decline largely represents elimination. The 2 phases can be separated out by "curve stripping" where the slope of the elimination phase is extrapolated back to zero time with intercept "B" on the y axis and the concentrations of this extrapolated line subtracted from the true plasma concentrations at each time point. This generates a series of residual values which can be plotted on the graph to describe the distribution process with slope "d" and intercept "D" on the y axis (Fig. 2.9).

Similarly, when a drug is administered orally and its plasma concentration-time profile described according to a 1 compartment model, the slope of the terminal linear phase can be extrapolated to zero (Fig. 2.10). Subtraction of the true plasma concentration-time values in the absorptive phase from the corresponding concentration-time values on the extrapolated line yields a series of residual concentrations which represent the absorptive phase (Gibaldi and Perrier,1982).

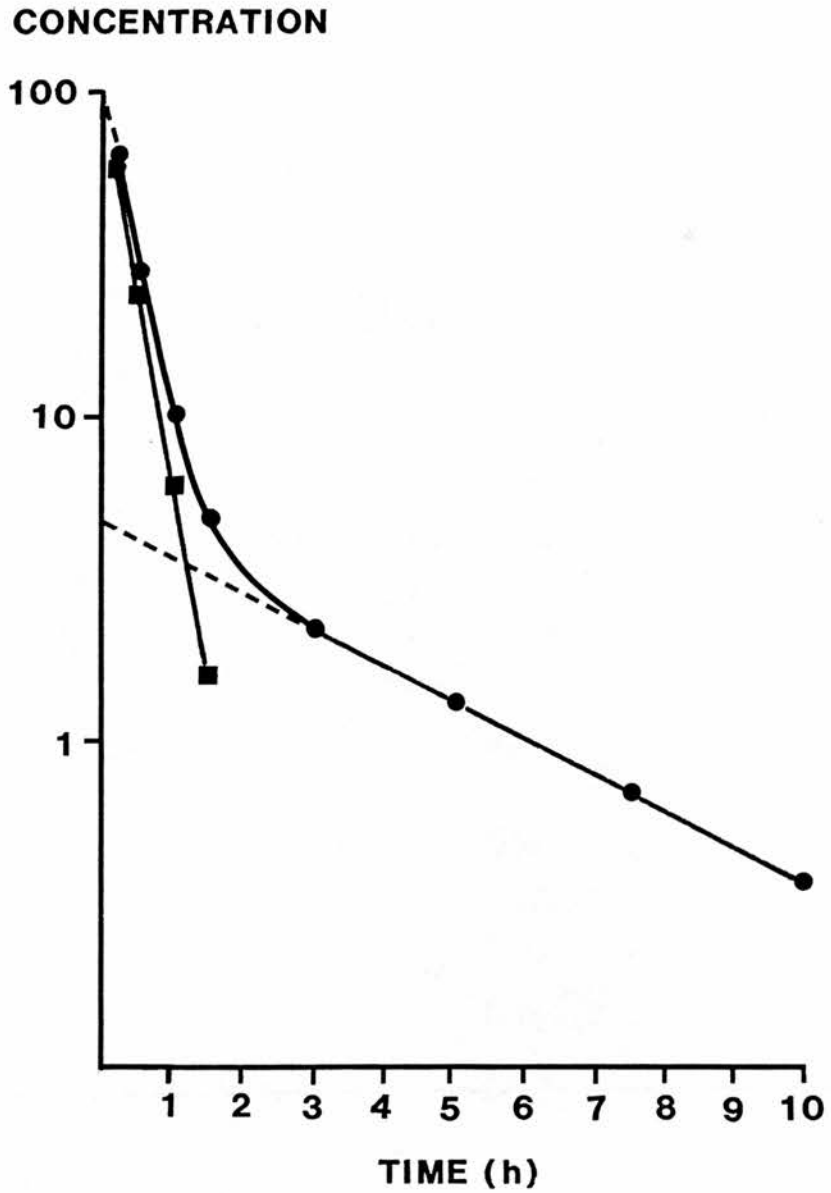


Fig 2.9. The log plasma concentration-time curve obtained following a rapid intravenous injection comprising a rapid distribution (d) and a slower elimination (ß) phase. The 2 phases can be separated out by "peeling" where the slope of the elimination phase is extrapolated back to zero time with intercept "B" on the y axis. The concentrations of this extrapolated line are subtracted from the true plasma concentrations at each time point and a series of residual values (▪) are generated to describe the distribution process with slope "d" and intercept "D" on the y axis (adapted from Gibaldi and Perrier, 1982).

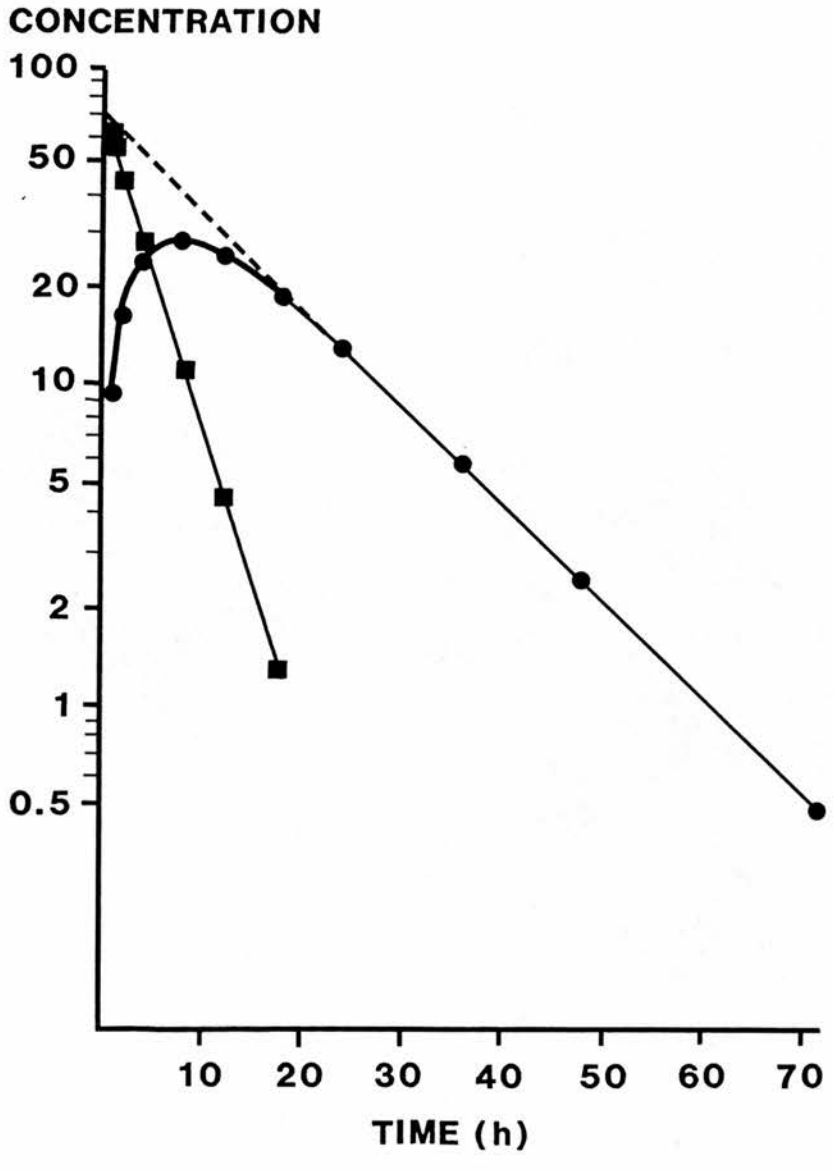


Fig 2.10. The log plasma concentration-time curve following oral administration of a drug described according to a 1 compartment model. The slope of the terminal linear phase can be extrapolated back to zero and the true plasma concentrations in the absorptive phase subtracted from the values on the extrapolated line yielding a series of residual concentrations (▪) which represents the absorptive phase.

Using the plot of log plasma concentration against time several important variables can be estimated.

(1) C_{max}

C_{max} represents the maximum concentration of drug in the plasma following an extravascular dose.

(2) T_{max}

T_{max} represents the time to reach peak plasma concentrations.

(3) $t_{½}$

The drug elimination half-life ($t_{½}$) is defined as the time taken for plasma concentrations to fall by 50% during the linear terminal elimination phase and is calculated as

$$t_{½} = \frac{\ln 2}{K} \qquad \text{Equation 2.2}$$

where K, the elimination rate constant of a drug is the fractional rate of decline per unit time and ln2 is the natural logarithm of 2 i.e. 0.693 (Gibaldi and Perrier, 1982).

(4) Area under the plasma concentration versus time curve (AUC).

This represents the area under a drug concentration-time plot and it can be calculated by the trapezoidal rule (Gibaldi and Perrier, 1982). This method involves the description of a given plasma concentration-time curve by a function that depicts the curve as a series of straight lines thereby enabling the area under the curve to be divided into a number of trapezoids (Fig. 2.11). The area of each trapezoid is calculated and summed. The area from the last experimental point C_n to infinity, ∞, (the "tail") is estimated as C_n/K.

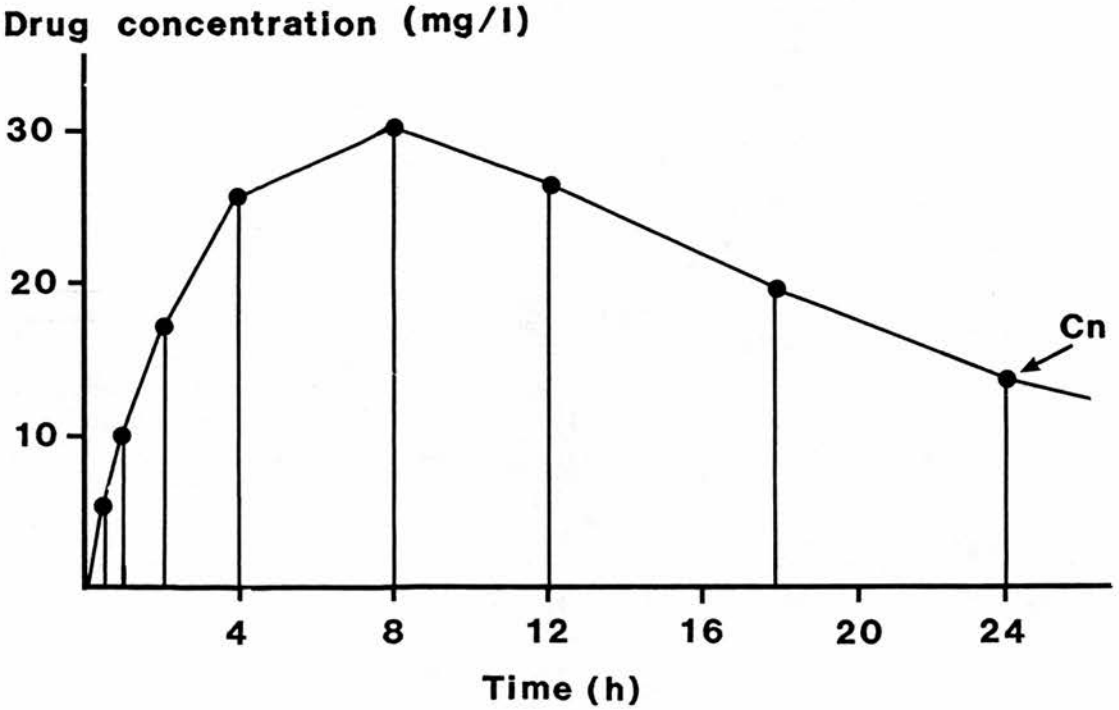


Fig 2.11. The area under the plasma concentration versus time curve (AUC) represents the area under a drug concentration-time plot as a series of trapezoids. The area of each trapezoid is calculated and summed and the area from the last experimental point C_n to infinity is estimated as C_n/K.

Thus, the $AUC_{0-\infty}$ = the sum of trapezoidal areas + $\frac{C_n}{K}$ Equation 2.3

(5) Volume of distribution (V_d).

The extent to which drugs are distributed or taken up into tissues is reflected by the apparent volume of distribution which can be considered as the volume into which the amount of drug in the body at any time would have to be diluted to give the observed plasma concentration in a 1 compartment model.

$$V_d = \frac{\text{amount of drug in body}}{\text{plasma concentration}} \qquad \text{Equation 2.4}$$

In a 2 or more compartment model the V_d is determined by the partitioning characteristics of the compound as well and it can be estimated as

$$V_d = \frac{Cl}{K} \qquad \text{Equation 2.5}$$

where Cl is the clearance of the drug (see below). Both methods require that the drug be given intravenously so that the amount reaching the systemic circulation is known and equivalent to the administered dose and be known (Gibaldi and Perrier, 1982).

(6) Clearance.

The term "clearance" refers to the removal of drug from the blood as it passes through an organ of elimination and it is defined as the volume of blood or plasma from which the drug is totally removed per unit time. The total clearance is the sum of the individual clearances that comprise drug elimination by parallel pathways (e.g. liver metabolism and renal excretion) and it can be calculated as:

$$\text{Total Cl} = \frac{\text{intravenous dose}}{AUC_{0-\infty}} \qquad \text{Equation 2.6}$$

The renal clearance of a drug can by calculated as follows:

$$\text{Renal Cl} = \frac{\text{amount excreted in a time interval}}{\text{AUC in the same interval}} \qquad \text{Equation 2.7}$$

Patients with end-stage renal failure who depend on dialysis for elimination of waste products may also clear drugs by this mechanism. Thus, in patients on peritoneal dialysis the peritoneal clearance of a drug can be calculated as

$$\text{peritoneal Cl} = \frac{\text{amount excreted into the peritoneum}}{\text{AUC in the same time interval}} \qquad \text{Equation 2.8}$$

Clearance of a drug by haemodialysis may be measured in terms of plasma $(Cl_d)_p$ and is defined as:

$$(Cl_d)_p = Q_p \frac{C_{ap} - C_{vp}}{C_{ap}} \qquad \text{Equation 2.9}$$

where Q_p is the plasma flow through the dialyser

C_{ap} is the "arterial" plasma concentration of the drug

C_{vp} is the "venous" plasma concentration of the drug, i.e. the plasma concentrations of drug entering and leaving the dialyser (Gibaldi and Perrier, 1982).

(7) Bioavailability

Bioavailability refers to the rate and extent of drug absorption (Gibaldi and Perrier, 1982). The proportion of unchanged drug that reaches the systemic circulation following extravascular administration (F) can be calulated as

$$F = \frac{AUC_{(o-\infty)} \text{ after an oral dose}}{AUC_{(o-\infty)} \text{ after the same intravenous dose}} \qquad \text{Equation 2.10}$$

The "SIPHAR" pharmacokinetic programme

The most practical way of analysing pharmacokinetic data is to use a computer with a programme such as "SIPHAR" (SIMED, 1988) for fitting experimental plasma concentration-time data to the chosen data. The plasma concentrations were entered in a data base, the results displayed graphically as a semi-log plot of drug concentration versus time and the number of exponentials considered suitable to describe the data were chosen by visual inspection. A theoretical curve was then superimposed on the experimental points according to the chosen model and the model parameters estimated by "peeling" (SIPHAR users manual, 1988).

Drugs were administered orally or by intravenous infusion over 1 h. Different equations were used to describe the plasma concentration-time curves depending on the routes of administration and selection of a 1 or 2 compartment model. Following oral administration a 1 compartment model with 2 phases was defined by the equation:

$$C_t = -Ae^{-kat} + Be^{-Kt} \qquad \text{Equation 2.11}$$

where C_t = the plasma concentration at time "t"

A = intercept on the y axis of the exponential representing absorption

B = intercept on y axis representing elimination

e = the base of the natural logarithm

K = the elimination rate constant of the drug

k_a = The absorption rate constant of the drug (Gibaldi and Perrier, 1982).

The corresponding equation for a 2 compartment model was:

$$C_t = -Ae^{-kat} + De^{-kdt} + Be^{-Kt} \qquad \text{Equation 2.12}$$

where D = intercept on the y axis representing distribution

kd = the rate constant of distribution

When a drug was administered by intravenous infusion and the plasma concentration time curve was best described by a single compartment model, the following equation was used to calculate concentrations during the infusion (Gibaldi and Perrier, 1982).

$$C_t = \frac{B(1-e^{Kt})}{KT} \qquad \text{Equation 2.13}$$

where "T" = the duration of the infusion

For calculation of concentrations once the infusion was stopped, the following equation was used:-

$$C_t = \frac{B(1-e^{Kt})\, e^{-K(t-T)}}{KT} \qquad \text{Equation 2.14}$$

The corresponding equations for a 2 compartment model were:-

$$C_t = \frac{D(1-e^{-kdt})}{k_d T} + \frac{B(1-e^{-Kt})}{KT} \qquad \text{Equation 2.15}$$

when $t < T$ and

$$C_t = \frac{D(1-e^{-kdt})\, e^{-Kd(t-T)}}{kdT} + \frac{B\,(1-e^{Kt})\, e^{-K(t-T)}}{KT} \qquad \text{Equation 2.16}$$

when $t > T$ (Notari, 1987).

Using the unrefined model parameters obtained by "peeling" the SIPHAR programme then minimised the differences between the theoretical curves and the observed data by iterative non-linear regression analysis with weighting of the data according to the method of least squares. The sum of the squared differences between the experimental and computed values were minimised using the error variance as a weighting factor. The programme improved the initial parameters by iteration so that the function was minimised and the theoretical curve of best fit was imposed on the experimental points.

In order to assess the goodness of fit the programme computed the correlation coefficient between observed and theoretical values and the coefficients of variation of each parameter. A coefficient of variation of over 20 to 30% was considered unacceptable and the computed algorithim was revised using a different weighting. As different equations could be used to fit the same data, the programme used a number of statistical tests to select the best model such as the Akaike, Schwarz and Leonard criteria (SIMED. 1988).

From the derived "best fit" model the programme calculated the elimination $t_{½}$, the absorption, distribution and elimination rate constants and the $AUC_{0-\infty}$. The V_d and total clearance were calculated using Equations 2.5 and 2.6 respectively. The latter variables are model independent parameters and no assumptions are made about the number of compartments.

Multiple dose simulation and predictions of steady-state concentrations

The SIPHAR programme was also used to describe the kinetic behaviour of a drug during repeated administration. The theoretical maximum and minimum steady-state concentrations were computed as well as the time to reach steady-state. The drug kinetics were assumed to be linear and models with 1 or 2 exponentials were used.

In order to calculate the plasma concentrations which would be achieved at steady-state during repeated regular dosing, the superimposition principle was applied (Gibaldi and Perrier, 1982 and Wagner, 1975). This overlay technique is based on the assumptions that each dose acts independently of every other dose, that the rate and extent of absorption and clearance are the same for each dosing interval and that the kinetics are linear. A characterisation of the concentration-time profile after a single dose is required and using the derived coefficients and exponentials the programme displayed a simulated curve which depended on the initial dose, the dosing interval and the dose given at each interval. From this curve the minimum and maximum steady-state plasma concentrations could be obtained. The predicted steady-state concentrations of a drug could be compared with the actual concentrations observed.

Percent absorbed and unabsorbed time plots

A useful way of evaluating the rate of drug absorption following oral administration is the construction of a percent absorbed versus time plot The amount of drug absorbed into the systemic circulation at a given time "t" equals the sum of the drug in the body plus the cumulative amount of drug eliminated (i.e. by urinary excretion, metabolism or any other route). The percent of drug absorbed at time "t" is defined as the ratio between the cumulative amount of drug absorbed from time "0" to time "t" and the total amount absorbed. It may be used to characterise the rate but not the extent of absorption (Gibaldi and Perrier, 1982).

For a 1 compartment model a modification of the Wagner-Nelson method may be used to calculate the percent of drug absorbed (Wagner and Nelson, 1964). Thus,

$$F_t = \frac{AUC_{0-t} + C_t/K}{AUC_{0-\infty}} \qquad \text{Equation 2.17}$$

where F_t is the fraction of the dose absorbed at time "t". The equation therefore re-

lates the cumulative amount of drug absorbed after a certain time to the amount of drug ultimately absorbed. Following an oral dose of a drug, the fraction absorbed at various time points can be calculated once the elimination rate constant is known.

Statistics

The following statistical tests were used to determine whether observed differences between groups were significant (Kirkwood, 1990).

Comparison of 2 groups

The Students "t" test was used to test whether the differences between a 2 groups of observations were significantly different and the level of significance was taken as $P < 0.05$ %. The unpaired "t" test was used when the 2 sets of measurements were completely independent and the paired "t" test was used when they were related to each other in some way such as repeated observations on the same subjects.

When the t test is used it is assumed that the data are normally distributed and that the populations from which the samples are drawn share the same variances. These assumptions characterise the parametric tests. If these conditions were not met or there was uncertainty, the non-parametric Mann-Whitney U test was used for unpaired data and the Wilcoxon signed rank test for paired samples (Kirkwood, 1990).

Comparison of several groups

One-way analysis of variance (ANOVA) was used to compare the means of several groups defined by a single factor. The method is based on how much of the overall variation in the data is attributable to differences between the group means and comparing this with differences between individuals in the same group (Kirkwood, 1990). Two-way ANOVA was used for data classified in 2 ways.

CHAPTER THREE

THE DISPOSITION OF PARACETAMOL FOLLOWING A SINGLE DOSE IN PATIENTS WITH END-STAGE RENAL FAILURE MAINTAINED ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD)

SECTION 3.1: INTRODUCTION

Continuous ambulatory peritoneal dialysis (CAPD) has become an established form of renal replacement therapy since its introduction in 1976 (Popovich et al., 1978). Pharmacokinetic studies in CAPD patients were stimulated early by the need to treat peritonitis with antibiotics given intraperitoneally but the disposition of drugs taken regularly by the patients for other diseases has not been studied in detail (Keller et al., 1990). The need for adjustment of drug dosage in patients with end-stage renal failure is well recognised (Bennett et al., 1983). However little attention has been paid to the extent to which peritoneal dialysis alters drug disposition (Paton et al., 1985).

Paracetamol is commonly used by patients with chronic renal failure because it is considered to be a relatively safe analgesic. In healthy volunteers about 90 % of a therapeutic dose is excreted in the urine within 24 h, predominantly as the glucuronide and sulphate conjugate of paracetamol with small amounts of glutathione-derived conjugates (Forrest et al., 1982). Following the administration of 1 g paracetamol to conservatively managed patients with chronic renal failure the concentrations of paracetamol glucuronide and sulphate were greatly increased compared to healthy volunteers given the same dose. Furthermore, in patients on haemodialysis studied on an inter-dialysis day, the concentrations were even higher (Prescott et al., 1989).

In CAPD patients taking paracetamol regularly marked accumulation of the conjugates would therefore be expected unless the peritoneal membrane was acting as an efficient elimination mechanism. Although peritoneal dialysis has been shown to be an effective method for removal of uraemic waste products it is thought to contribute little to the clearance of most drugs (Paton et al., 1985). In addition little work has been done on the effect of dialysate clearance on the elimination of drug metabolites but work with metronidazole and cefotaxime suggests little clearance of their metabolites during

CAPD (Guay et al., 1984 and Alexander et al., 1984). The glucuronide conjugate of paracetamol was isolated however in the dialysate of patients on CAPD (Schoots et al., 1990).

The object of this study was to establish the disposition of a single dose of paracetamol in patients with end-stage renal failure maintained on CAPD. In particular it was hoped to establish the extent of peritoneal clearance of paracetamol and its metabolites.

SECTION 3.2: METHODS

Patients

Six patients (1 female, 5 male) maintained on CAPD for end-stage renal failure (creatinine clearance < 5 ml.min^{-1}) were studied. Their mean age was 55 yr (range 31-71) and weight 68 kg (range 49-84). Details of the medical histories and medication are given in Table 3.1 and results of haematology and biochemistry screening in Table 3.2. Patients with a history of peritonitis in the previous 3 months were excluded as were patients with a haemoglobin concentration of less than 7 g.dl^{-1}. Patients were asked to avoid taking paracetamol for the 7 days before the study.

Patients performed 3 or 4 peritoneal exchanges each day via indwelling Tenckhoff catheters at approximately 08.00, 13.00, 18.00 and 23.00 h as described in Chapter 2. Details of the dialysis regimes and daily fluid allowances are given in Table 3.3.

Table 3.1. Clinical details of six patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis (CAPD) who were given 1 g paracetamol orally.

Patient No.	Age & sex	Medical diagnosis	Regular drug therapy
1	62 M	Goodpasture's Syndrome hypertension	"Fefol vit"* 2 daily
2	65 M	diabetes mellitus type 2 hypertension chronic renal failure ?cause	gliclazide 80 mg daily metoprolol 25 mg BD digoxin 62.5 μg daily aluminium hydroxide 475 mg TID "Fefol Vit" 2 daily
3	52 M	diabetes mellitus type 2 diabetic nephropathy	gliquidone 30 mg BD nifedipine retard 10 mg BD metoprolol 25 mg BD quinine sulphate 300 mg nocte aluminium hydroxide 950 mg TID
4	48 M	glomerulonephritis failed transplant hypertension ischaemic heart disease	isosorbide mononitrate 10 mg daily metoprolol 25 mg BD terfenadine 60 mg BD prednisolone 2 mg daily aluminium hydroxide 475 mg TID
5	31 F	pyelonephritis hypoplastic right kidney failed transplant hypertension	nifedipine 5 mg BD metoprolol 50 mg BD doxazosin 4 mg BD "Fefol Vit" 2 daily aluminium hydroxide 950 mg BD calcium carbonate 1.26 g daily
6	71 M	proliferative glomerulonephritis ischaemic heart disease hypertension	diltiazem 60 mg TID captopril 25 mg BD prazosin 2 mg TID aluminium hydroxide 475 mg TID

* ferrous sulphate 150 mg with vitamins B group and C

Table 3.2. Clinical biochemical and haematological blood test results in 6 patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis given 1.0 g of paracetamol orally.

Plasma concentration	Patients 1	2	3	4	5	6	Normal range
protein $g.l^{-1}$	68	66	66	57	55	72	60-80
albumin $g.l^{-1}$	38	39	38	34	34	42	36-47
calcium $mmol.l^{-1}$	2.2	2.3	2.4	2.1	2.3	2.1	2.1-2.6
phosphate $mmol.l^{-1}$	2.0	1.7	1.8	1.5	1.8	1.9	0.8-1.4
alkaline phosphatase $u.l^{-1}$	99	124	76	71	419	137	40-100
bilirubin $\mu mol.l^{-1}$	4	10	7	4	4	6	2-17
alanine amino-transferase $u.l^{-1}$ (ALT)	16	35	24	16	10	14	10-40
gamma-glutamyl transferase $u.l^{-1}$ (GGT)	10	53	25	9	10	27	10-55
bicarbonate $mmol.l^{-1}$	18	20	23	20	23	20	24-30
creatinine $\mu mol.l^{-1}$	999	1326	1163	1128	1125	1233	55-150
urea $mmol.l^{-1}$	20.1	28.9	26.6	21.8	23.2	20.9	2.5-6.6
haemoglobin $g.dl^{-1}$	8.4	8.7	8.6	10.3	5.8	9.6	13-18
white blood cells $x\ 10^{9}.l^{-1}$	5.6	6.9	7.0	5.2	5.0	6.0	4-11
platelets $x\ 10^{9}.l^{-1}$	253	223	181	268	169	196	150-350

Table 3:3. Details of the daily fluid allowance and dialysis regimes of six patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis given 1 g paracetamol orally.

Patient No.	Daily fluid intake (ml)	Time on CAPD	Exchanges per day	Glucose content of each bag (%)
1	750	3 months	3	2.27 x 1 1.36 x 2
2	1500	3 years	4	3.86 x 1 2.27 x 3
3	800	18 months	4	1.36 X 4
4	750	6 months	4	2.27 x 3 1.36 x 1
5	750	6 months	4	2.27 x 3 1.36 x 1
6	1000	2 years	4	2.27 x 2 1.36 x 2

Experimental design

On the morning of the study day the fasting patients came to the CAPD training room at 08.30 h with their overnight dialysate still in the peritoneal cavity. The weight and glucose content of a new bag of dialysate was noted and the patients then performed the first exchange of the day. An intravenous cannula was inserted in a forearm vein and 1 g of paracetamol was administered orally (2 x 500 mg soluble "Panadol" tablets) dissolved in approximately 100 ml of water. The patients then remained recumbent for 1 h.

The patients were allowed their normal fluid intake and they regulated this themselves throughout the day (Table 3.3). Breakfast was served one hour after the dose of paracetamol when the patients took their normal medications (Table 3.1) and lunch was taken at 13.00 h according to their usual dietary requirements.

Venous blood (3 ml) was sampled before the paracetamol was administered and then at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes after the dose. The 3 or 4 used dialysate bags of that day were kept and the volume measured. All urine passed during the 24 h period was collected. The patients were free to go home after their 3rd exchange of the day at approximately 17.30 h.

Samples

All samples were processed as described in Chapter 2.

Drug assay

Plasma, urine and dialysate concentrations of paracetamol and its conjugates were measured by HPLC as described in Chapter 2.

Pharmacokinetic analysis of data

The SIPHAR pharmacokinetic programme was used to analyse the plasma concentration-time curves of paracetamol as described in Chapter 2. The data were best described by a two compartment model with 3 phases comprising an absorption and distribution phase followed by an elimination phase from 2 to 8 h. Initial parameters were estimated by peeling and refined by iterative analysis. The goodness of the fit was established by comparing the coefficients of variation of each parameter as described in Chapter 2. These are listed in Table 3.4 and were less than 20 to 30 % in all cases.

Two of the patients (4 and 5) had peaks corresponding to paracetamol glucuronide and sulphate in the plasma sampled at time 0 before the dose of paracetamol. This was confirmed by the addition of glucuronidase and sulphatase as described in Chapter 2. The corrected plasma concentrations of both conjugates were then calculated using Equation 2.1 as described in Chapter 2.

The fitted model was used to describe the disposition of oral paracetamol and to estimate the elimination half life. The $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}\infty}$ of paracetamol and the $AUC_{0\text{-}8h}$ of its glucuronide and sulphate conjugates was calculated using the trapezoidal rule. The peritoneal clearances of paracetamol and paracetamol glucuronide and sulphate were calculated as described in Chapter 2.

Table 3.4. The coefficients of variation (CV%) for the estimated parameters obtained by analysis of the plasma concentration-time data following the administration of 1 g paracetamol to patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis. The data were best described by a two compartment model comprising absorption, distribution and elimination phases. Initial parameters were estimated by peeling and refined by iterative analysis. Each phase is represented by an intercept and slope as described in Chapter 2.

Patient No.	Absorption		Distribution		Elimination	
	Intercept (CV%)	slope (CV%)	Intercept (CV%)	slope (CV%)	Intercept (CV%)	slope (CV%)
1	0.6	21.0	13.3	21.3	4.0	3.0
2	0.0	28.1	1.4	4.7	2.1	3.1
3	0.8	14.4	16.0	17.2	3.0	2.1
4	0.1	9.8	0.6	9.1	5.8	4.5
5	0.2	23.9	7.4	7.0	4.1	1.9
6	0.2	31.2	8.5	20.2	6.3	4.1

SECTION 3.3: RESULTS

Paracetamol

The individual and mean plasma concentrations of paracetamol following the administration of 1 g orally to the 6 CAPD patients are shown in Figs. 3.1 and 3.2 respectively and the estimated pharmacokinetic parameters are listed in Table 3.5. Paracetamol was absorbed rapidly with mean peak plasma concentrations of 15.9 $\pm$3.6 mg.l^{-1} occurring at 27.5 $\pm$5.6 min after the dose (Table 3.5). The $AUC_{0\text{-}8h}$ was 2551 $\pm$400 min.mg.l^{-1} and $AUC_{0\text{-}\infty}$ was 2998 $\pm$634 min.mg.l^{-1}. The mean elimination half life of paracetamol derived from the fitted model was 180.3 $\pm$6.2 min (Table 3.5).

Paracetamol glucuronide conjugate

The individual and mean plasma concentrations of the glucuronide conjugate of paracetamol are shown in Figs. 3.3 and 3.2 respectively and the pharmacokinetic parameters are listed in Table 3.6. Following the administration of paracetamol to the CAPD patients the mean C_{max} of its glucuronide conjugate was 24.2 $\pm$6.5 mg.l^{-1} and the mean T_{max} was 320.0 $\pm$89.4 min. The mean $AUC_{0\text{-}8h}$ was 7623 $\pm$2635 min.mg.l^{-1}. The plasma concentrations of the glucuronide conjugate varied little from 2 to 8 h and it was not possible therefore to accurately estimate the elimination half life (Fig 3.2).

Paracetamol sulphate conjugate

The individual plasma concentrations of the sulphate conjugate of paracetamol are shown in Fig 3.3 and the pharmacokinetic parameters are listed in Table 3.6. Following the administration of paracetamol to the CAPD patients the mean C_{max} of the sulphate conjugate was 14.1 $\pm$3.5 mg.l^{-1} and the mean T_{max} was 290.0 $\pm$144.6 min. The $AUC_{0\text{-}8h}$ was 4645 $\pm$1482 min.mg.l^{-1}. As in the case of the glucuronide conjugate it was not possible to estimate the elimination half life because the concentration of the sulphate conjugate varied little from 2 to 8 h (Figure 3.3).

Table 3.5. Maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the plasma concentration time curve from 0 to 8 h and from 0 h to infinity (AUC_{0-8h} and $AUC_{0-\infty}$) and elimination half life ($t_{½}$) of paracetamol in 6 patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis (CAPD) given 1 g paracetamol orally.

Patient No.	C_{max} ($mg.l^{-1}$)	T_{max} (mins)	AUC_{0-8} ($mg.min.l^{-1}$)	$AUC_{0-\infty}$ ($mg.min.l^{-1}$)	$T_{½}$ (mins)
1	12.7	30	2410	2790	160.3
2	16.8	15	3214	4619	318.1
3	12.5	30	2446	2809	166.5
4	21.1	30	2507	2831	162.7
5	19.8	30	2822	3182	132.0
6	12.6	30	1906	2140	142.0

Table 3.6. Maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the plasma concentration time curve from 0 to 8 h (AUC_{0-8}) of the glucuronide (G) and sulphate (S) conjugates of paracetamol and the ratios of the AUC_{0-8h} of the G and S conjugates in 6 patients with end stage renal failure given 1 g paracetamol orally.

Patient No.		C_{max} (mg)	T_{max} (mins)	AUC_{0-8h} ($mg.min.l^{-1}$)	Ratio AUC_{0-8h} G : S
1	G	32.2	300	11117	2.6
	S	13.8	180	4191	
2	G	14.6	420	4768	0.9
	S	13.9	480	5271	
3	G	21.4	360	7102	1.3
	S	13.9	360	5308	
4	G	24.5	180	6252	3.1
	S	8.4	420	1994	
5	G	19.8	240	5215	1.2
	S	14.4	240	4233	
6	G	32.5	420	11281	1.6
	S	20.4	60	6871	

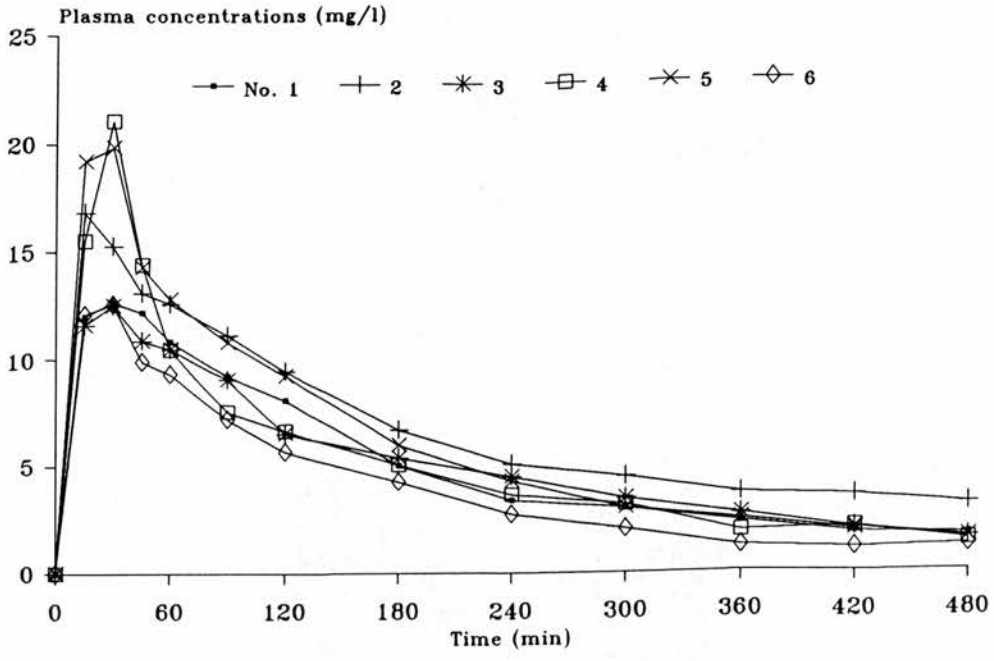


Fig 3.1. Plasma concentrations of paracetamol in 6 CAPD patients following 1 g orally.

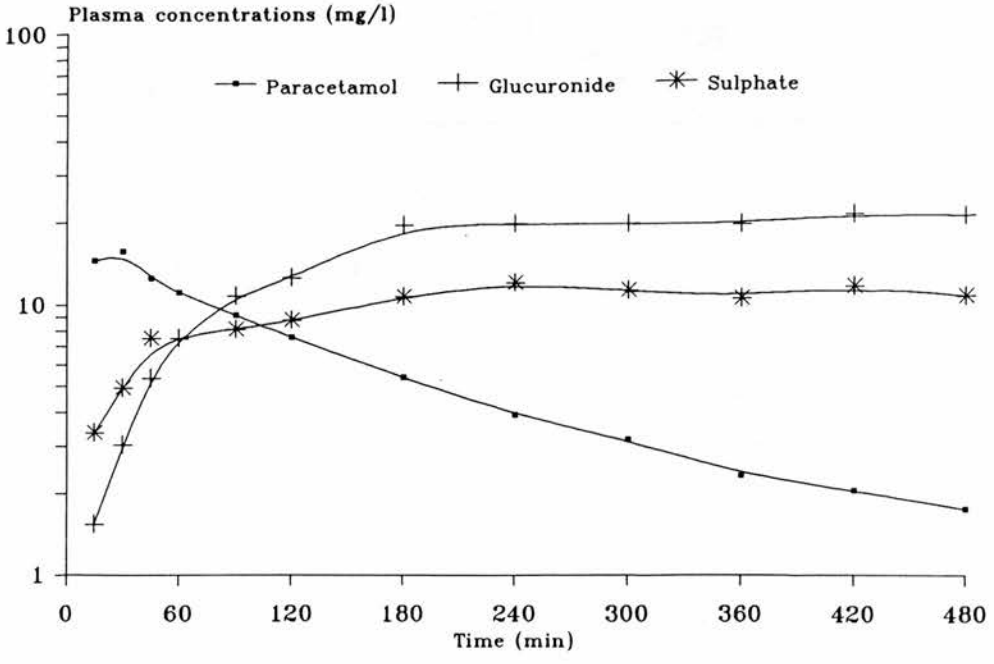


Fig 3.2. Mean plasma concentrations of paracetamol and its glucuronide and sulphate conjugates in 6 CAPD patients following 1 g oral paracetamol.

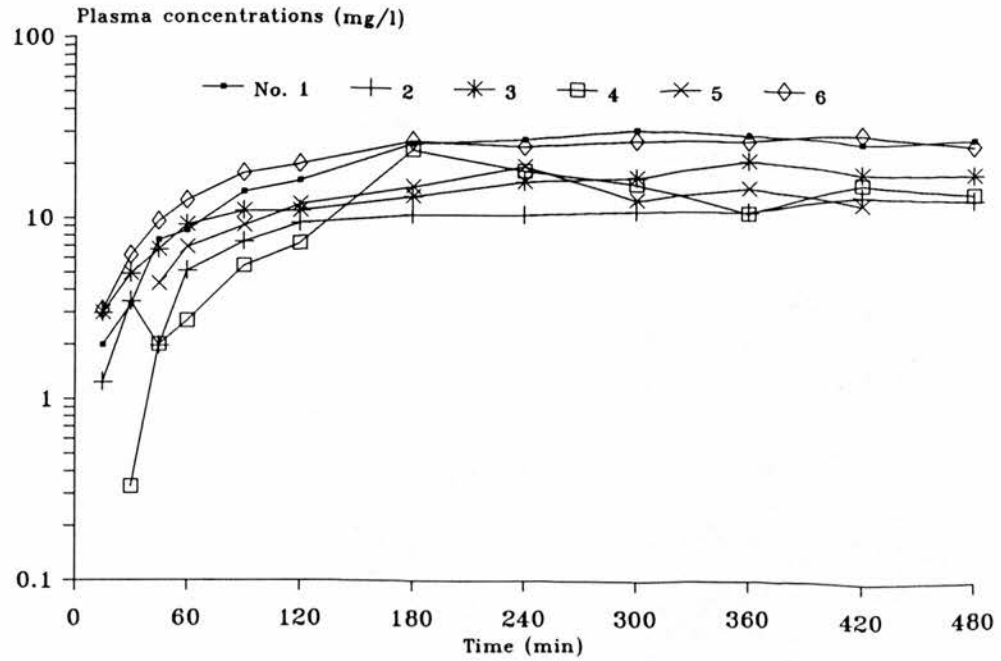


Fig 3.3. Plasma concentrations of paracetamol glucuronide in 6 CAPD patients following the administration of 1 g paracetamol orally.

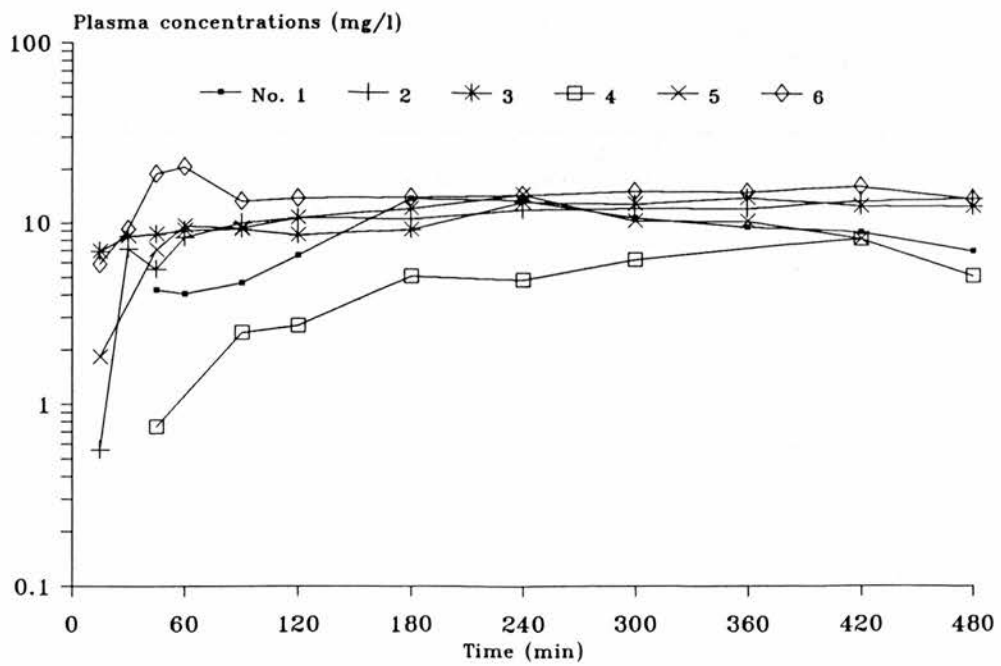


Fig 3.4. Plasma concentrations of paracetamol sulphate in 6 CAPD patients following the administration of 1 g paracetamol orally.

Normally paracetamol is metabolised to its glucuronide and sulphate conjugate in a ratio of 60 to 30 %. This ratio is unchanged in patients with conservatively managed renal impairment (Prescott et al., 1989). In the present study although the mean ratio of the AUC_{0-8h} of the glucuronide and sulphate conjugates was 1.8:1, 3 of the 6 patients had lower ratios than expected (Table 3.6)

Paracetamol cysteine and mercapturate conjugates

The cysteine and mercapturate acid conjugates could not be measured in the plasma of the CAPD patients.

Urinary recovery of paracetamol and its glucuronide and sulphate conjugates

Three of the 6 patients were anuric (2, 4 and 5). Only one patient (1) had measurable amounts of paracetamol in the urine in the 24 h following the dose (1.6 mg). The mean 24 h urinary recoveries of the glucuronide and sulphate conjugates in the 3 patients were 39.2 $\pm$15.5 mg and 11.8 $\pm$4.8 mg respectively.

Recovery of paracetamol and its conjugates in the dialysate

The recovery of paracetamol and its glucuronide and sulphate conjugates during each exchange is shown in Table 5.7. Only 2 of the 3 used dialysate bags were collected from patient 1. Significant amounts of paracetamol were recovered in the first two exchanges only. The total amount recovered was 16.0 $\pm$7.5 mg or 1.6 $\pm$0.7 % of the administered dose. The corresponding peritoneal clearance from 0 to 8 h was 6.0 $\pm$2.1 $ml.min^{-1}$.

The recovery of the glucuronide conjugate in the dialysate was 69.4 $\pm$33.4 mg in the 8 h following the dose of paracetamol and the corresponding peritoneal clearance was 6.8 $\pm$2.0 $ml.min^{-1}$. The total recovery in the dialysate in 24 h was 165.2 $\pm$63.0 mg (n=5).

Table 3.7. The recovery of paracetamol and its glucuronide and sulphate conjugates in the dialysate of 6 patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis (CAPD). Patients performed 3 (patient 1) or 4 peritoneal exchanges a day starting at approximately 08.30, 13.00, 18.00 and 23.00 h. Paracetamol 1 g was administered orally at the start of the first exchange at 08.30 h.

Paracetamol recovery (mg)

	Patient					
	1	2	3	4	5	6
Exchange						
1	14.1	15.2	10.0	10.5	10.0	8.2
2	2.8	13.8	8.6	3.8	0.0	0.0
3	-	0.0	0.0	0.0	0.0	0.0
4		0.0	0.0	0.0	0.0	0.0

Paracetamol glucuronide recovery (mg)

	Patient					
	1	2	3	4	5	6
Exchange						
1	25.9	19.6	8.0	27.2	42.0	30.8
2	44.1	25.3	19.4	40.1	82.6	51.9
3	-	28.3	26.0	59.8	108.5	52.0
4		33.1	36.1	52.9	32.4	49.8

Paracetamol sulphate recovery (mg)

	Patient					
	1	2	3	4	5	6
Exchange						
1	0.0	13.1	6.0	14.6	0.0	0.0
2	0.0	18.4	10.8	18.9	18.9	0.0
3	-	17.4	12.0	23.4	2.2	0.0
4		18.6	14.0	18.4	0.0	0.0

Following the dose of paracetamol 27.3 ±7.4 mg (n=3) of the sulphate conjugate had been recovered in the dialysate by the end of the second exchange at 8 h. No sulphate conjugate was recovered in the dialysate of patient 1 or 6 during that time or from the 1st and 4th exchange of patient 4 possibly due to technical problems. In the other 3 patients, the mean peritoneal clearance from 0 to 8 h was 4.7 ±1.2 $ml.min^{-1}$. The total recovery in the dialysate in the 24 h period was 61.9 ±13.8 mg (n=3).

Assuming that paracetamol is metabolised to its glucuronide and sulphate metabolite in the normal ratio of 2:1 in the CAPD patients and that the absorption of paracetamol was complete, 27.5 ±10.5 % of the total amount of the glucuronide conjugate formed and 20.6 ±13.8 % (n=3) of the sulphate conjugate formed were removed in the dialysate in the 24 h following the dose of paracetamol. The concentrations of the cysteine or mercapturate conjugates were too low to be measured in the dialysate.

SECTION 3.4: DISCUSSION

In patients with end-stage renal failure maintained on CAPD, the absorption of paracetamol was rapid and maximum concentrations were achieved within 30 min. Mean maximum plasma concentrations of paracetamol in fasting healthy subjects have been noted at 22 min following the ingestion of paracetamol in solution (Nimmo et al., 1975) and at 20 min (McGilveray and Mattok, 1972), 21 min (Prescott et al., 1989), 60 min (Dordoni et al., 1973) and 1.4 h (Heading et al., 1973) after the ingestion of paracetamol tablets.

The absorption of paracetamol has previously been shown to be normal in patients with conservatively managed renal failure and haemodialysis patients (Prescott et al., 1989) and maximum plasma concentrations and the time taken to achieve them were similar to the findings in the present study. Similarly, the elimination half life of

paracetamol from 2 to 8 h in healthy volunteers, conservatively managed patients with chronic renal failure and haemodialysis patients was in agreement with the elimination half life of 3 h in the present study.

Following a therapeutic dose, paracetamol is extensively metabolised to its glucuronide and sulphate conjugate in the liver (Forrest et al., 1982). In healthy fasting volunteers given 1 g orally peak plasma concentrations of the glucuronide conjugate of 9.4 $\pm$2.6 mg.l^{-1} occurred 2.0 $\pm$4.4 h after the dose (Prescott et al., 1989). The corresponding concentrations of the sulphate conjugate were 3.7 $\pm$0.9 mg.l^{-1} and these occurred at 1.1 $\pm$0.5 h. In the present study not only were the concentrations of the glucuronide and sulphate conjugates much higher, but the mean times to reach maximum concentration were delayed for 5.3 $\pm$1.5 h and 4.8 $\pm$2.4 h respectively. Similar results were found in conservatively managed patients with chronic renal failure with residual creatinine clearances of 5 to 23 ml.min^{-1} (Prescott et al. 1989). Furthermore, although the relative amounts of glucuronide to sulphate conjugates formed was similar to normal in the CAPD patients as judged by the mean ratios of the $AUC_{0\text{-}8h}$, the ratio was reduced in some of the patients.

Less than 2 % of the administered dose of paracetamol was recovered in the dialysate and the mean peritoneal clearance was 6 ml.min^{-1}. The peritoneal clearance of the glucuronide and sulphate conjugate were similar and less than 30 % of the conjugates formed were recovered in the dialysate in 24 h, assuming that absorption of paracetamol was complete and that approximately 60 % and 30 % of the dose was metabolised to the glucuronide and sulphate conjugate respectively.

The factors which affect the rate of removal of a drug during peritoneal dialysis are thought to be the physiochemical properties of the drug (including the molecular weight, water solubility and lipid partition), the physiology of the peritoneal mem-

brane (surface area, vascularity and ultrafiltration properties of the membrane) and the peritoneal solution type, flow rate and osmolality (Paton et al., 1985). In addition, the pharmacokinetic properties of a drug (or drug metabolite) which affect its rate of removal are the degree to which it is protein bound and its volume of distribution (Paton et al., 1985). The greatest contribution to clearance by the peritoneum is thought to be for drugs which are almost exclusively removed by the kidney (Paton et al., 1985 and Keller et al., 1990). In order for a drug to be cleared by the peritoneal membrane to a significant degree it has therefore to have low plasma protein binding (<20 %), a small volume of distribution (<1 l.kg^{-1}) and little non-renal clearance under normal circumstances. The low flow rate of the peritoneal effluent (7ml.min^{-1}) appears to be the most important limiting factor for the extraction capacity of CAPD (Keller et al., 1989).

The highly polar glucuronide and sulphate conjugates of paracetamol are normally excreted by the kidneys probably both by filtration at the glomerulus and active secretion at the tubules resulting in clearances of about 130 and 170 ml.min^{-1} respectively (Forrest et al., 1982). The volumes of distribution of the glucuronide and sulphate conjugate of paracetamol are small (<0.3 l.kg^{-1}) and are similar in healthy volunteers and patients with chronic renal failure (Prescott et al., 1989). Furthermore, the conjugates do not bind to plasma proteins even at the relatively high concentrations found in anephric patients (Lowenthal et al., 1976). It was not unexpected therefore that these patients with end-stage renal failure would eliminate some of the accumulated conjugates of paracetamol by peritoneal clearance but that the extraction capacity would be limited to a maximum of about 7ml.min^{-1}.

Although there is some evidence that the biliary excretion of paracetamol conjugates may be increased in renal failure (Siegers and Klaassen, 1984), CAPD patients are largely dependent on peritoneal clearance for elimination of paracetamol metabolites.

Given the low extraction capacity peritoneal dialysis following a single therapeutic dose of paracetamol, it would seem inevitable that marked accumulation of the glucuronide and sulphate metabolites would occur during regular dosing. Although these metabolites are thought to be inactive under normal circumstances their biological effects are unknown at very high concentrations (Prescott et al., 1989). In addition, some of the retained conjugates might undergo enterohepatic elimination with hydrolysis in the gut by microflora and subsequent reabsorption of the parent compound (Veerbeck et al., 1981 (a)). Furthermore the production of the glutathione-derived metabolites might be increased under such circumstances.

It seems inevitable therefore that patients with end-stage renal impairment who are maintained on CAPD will accumulate polar drug metabolites given the low extraction capacity of the peritoneal membrane. It may be necessary to reduce the dose when renal elimination of either the drugs themselves or their active or inactive metabolites is limited by renal disease as has been demonstrated for paracetamol.

CHAPTER FOUR

THE DISPOSITION OF PARACETAMOL AND THE ACCUMULATION OF ITS GLUCURONIDE AND SULPHATE CONJUGATES DURING MULTIPLE DOSING IN PATIENTS WITH CHRONIC RENAL FAILURE

SECTION 4.1: INTRODUCTION

The excretion of polar drug metabolites is impaired in patients with chronic renal failure. When single doses of paracetamol were given to patients with renal impairment, the plasma concentrations of its glucuronide and sulphate conjugates were greatly increased compared with those observed in healthy volunteers (Prescott et al., 1989). Similarly, as discussed in Chapter 3, patients with end-stage renal failure maintained on CAPD had very high plasma concentrations of these conjugates following a single dose of paracetamol and their clearance across the peritoneal membrane was limited by a low effective "flow rate". Furthermore, in anephric patients haemodialysis appeared to be the major route of elimination of paracetamol metabolites (Øie et al., 1975).

Therefore, when patients with moderate to severe renal impairment take paracetamol regularly over a long period of time, significant accumulation of its polar metabolites would be expected. Under these conditions it is possible that the retained conjugates might undergo enterohepatic circulation, with some regeneration of the parent compound by hydrolysis in the gastrointestinal tract with subsequent reabsorption (Verbeeck et al., 1981 (a)). It is also possible that the production of the potentially toxic glutathione-derived conjugates might be increased.

Few studies have been done to determine the degree of drug and metabolite accumulation during long term treatment of patients with impaired renal function. The disposition of paracetamol and its metabolites was therefore compared in healthy volunteers and in conservatively managed patients with chronic renal failure taking 1 g of paracetamol three times a day for 10 days. The plasma concentrations of the glucuronide and sulphate conjugates were compared with those expected on the basis of previous single-dose studies (Prescott et al., 1989).

SECTION 4.1: METHODS

Volunteers

Studies were carried out in 6 healthy volunteers (3 men and 3 women) of mean age 32 yr (range 18-37) and mean weight 60 kg (range 48-75). They had no medical illness, physical examination was normal, they were on no medication, they did not smoke and they all claimed to drink less than 5 units of alcohol per week. The results of routine biochemical and haematological screening tests are presented in Table 4.1.

Patients

Six patients (5 men, 1 woman) on conservative management for chronic renal failure were also studied. Their mean age and weight were 55 yr (range 43-75) and 74 kg (range 52-97). Details of the medical histories and medication are presented in Table 4.2 and the results of biochemistry and haematology screening in Table 4.3. The mean plasma creatinine concentration was 451.5 $\pm$178.8 μmol.l^{-1} and mean creatinine clearance 22.8 $\pm$10.6 ml.min^{-1}. Both patients and volunteers were asked to avoid taking paracetamol for 7 days before and for 4 days after the study.

Experimental Design

The patients and volunteers took paracetamol 1 g (2 x 500 mg soluble "Panadol" tablets) dissolved in approximately 100 ml of water, at 10.00, 16.00 and 22.00 h for 10 days. Food was avoided for 2 h before and for 2 h after dosing. Venous blood was sampled at 09.00 h on the first day of the study before starting paracetamol treatment, at the same time (09.00 h) each morning for the next 10 days, and on the mornings of the 2nd and 4th days after the last dose of paracetamol.

Samples

All samples were processed as described in Chapter 2.

Table 4.1. Clinical biochemical and haematological blood test results in 6 healthy volunteers given 1.0 g of paracetamol three times a day for 10 days.

Plasma concentration	Volunteers 1	2	3	4	5	6	Normal range
protein $g.l^{-1}$	70	74	71	68	70	71	60-80
albumin $g.l^{-1}$	40	50	45	45	43	48	36-47
calcium $mmol.l^{-1}$	2.4	2.5	2.3	2.3	2.4	2.4	2.1-2.6
phosphate $mmol.l^{-1}$	1.1	0.9	1.1	0.6	1.3	0.9	0.8-1.4
alkaline phosphatase $u.l^{-1}$	26	49	62	91	27	16	140-100
bilirubin $\mu mol.l^{-1}$	11	9	5	4	6	2	52-17
alanine amino-transferase $u.l^{-1}$ (ALT)	49	22	16	23	10	19	10-40
gamma-glutamyl transferase $u.l^{-1}$ (GGT)	11	14	8	15	8	16	10-55
bicarbonate $mmol.l^{-1}$	24	28	28	25	25	24	24-30
creatinine $\mu mol.l^{-1}$	82	85	75	96	77	64	55-150
urea $mmol.l^{-1}$	3.3	5.1	4.6	6.1	4.6	3.8	2.5-6.6
haemoglobin $g.dl^{-1}$	13.4	15.4	14.3	16.2	13.8	13.0	13-18
white blood $x\ 10^{9}.l^{-1}$	7.7	4.4	4.9	7.2	7.3	5.3	4-11
platelets $x\ 10^{9}.l^{-1}$	230	285	216	171	317	218	150-350

Table 4.2. Clinical details of six conservatively managed patients with chronic renal failure taking 1.0 g paracetamol three times a day for 10 days.

Patient No.	Age & sex	Medical diagnosis	Regular drug therapy
1	59 M	membranous glomerulnephritis hypertension	frusemide 40 mg daily enalapril 2.5 mg daily aspirin 75 mg daily sodium bicarbonate 1.5 g TID
2	40 M	Diabetic nephropathy insulin dependent diabetes mellitus hypertension	insulin captopril 25 mg TID frusemide 40 mg daily metoprolol 50 mg BD
3	43 F	pyelonephritis	sodium bicarbonate 1 g QID aluminium hydroxide 950 mg QID 1α-hydroxycholecalciferol 0.25 μg daily clonazepam 1 mg nocte
4	50 M	mesangial proliferative glomerulonephritis hypertension	nifedepine retard 10 mg BD metoprolol 50 mg BD
5	75 M	pyelonephritis ileal loop diversion & total cystectomy for carcinona of bladder	isosorbide mononitrate 10 mg BD sodium bicarbonate 500 mg QID
6	65 M	diabetic nephropathy insulin dependent diabetes mellitus hypertension	insulin indapamide 2.5 mg daily enalapril 10 mg BD sodium bicarbonate 1 g TID

Table 4.3. Clinical biochemical and haematological blood test results in 6 conservatively managed patients with chronic renal failure taking 1.0 g of paracetamol three times a day for 10 days.

Plasma concentration	Patients 1	2	3	4	5	6	Normal range
protein $g.l^{-1}$	74	63	64	60	73	65	60-80
albumin $g.l^{-1}$	35	40	41	33	42	39	36-47
calcium $mmol.l^{-1}$	2.3	2.3	2.6	2.1	2.3	1.9	2.1-2.6
phosphate $mmol.l^{-1}$	1.0	1.5	2.3	0.8	0.9	1.5	0.8-1.4
alkaline phosphatase $u.l^{-1}$	74	89	167	73	105	135	40-100
bilirubin $\mu mol.l^{-1}$	4	11	5	6	6	6	2-17
alanine amino-transferase $u.l^{-1}$ (Alt)	29	27	19	20	10	31	10-40
gamma-glutamyl transferase $u.l^{-1}$ (GGT)	15	13	31	16	14	36	10-55
bicarbonate $mmol.l^{-1}$	15	19	21	23	25	21	24-30
creatinine $\mu mol.l^{-1}$	407	312	774	379	247	590	55-150
urea $mmol.l^{-1}$	15.4	20.2	21.2	14.1	14.0	29.4	2.5-6.6
creatinine clearance $ml.min^{-1}$	22	42	8	18	29	18	>100
haemoglobin $g.dl^{-1}$	12.6	13.7	10.7	11.7	12.2	10.2	13-18
white cells $x\ 10^{9}.l^{-1}$	6.2	8.7	5.6	7.4	8.9	4.8	4-11
platelets $x\ 10^{9}.l^{-1}$	153	273	263	306	249	197	150-350

Drug assay

Plasma concentrations of paracetamol and its conjugates were measured by HPLC as described in Chapter 2.

Pharmacokinetic analysis of data

Steady-state concentrations of paracetamol glucuronide and sulphate conjugates were predicted from the relationship between the degree of renal failure and the minimum steady-state concentrations calculated from data obtained in a previous single-dose study where a group of conservatively managed patients with chronic renal failure were given a single dose of 1 g paracetamol (Prescott et al., 1989). The data were fitted to a single compartment model by the SIPHAR pharmacokinetic programme as discussed in Chapter 2. The "dose" of conjugate was taken as the amount formed from the administered paracetamol.

Using the derived coefficients and exponents the minimum steady-state conjugate concentrations with the present repeated dose schedule were simulated according to the superimposition principle as described in Chapter 2 (Wagner 1975). The results are presented on Table 4.4 .

The calculated concentrations of glucuronide and sulphate conjugates were closely related to the plasma creatinine concentration ($r = 0.89$ and 0.95 respectively, $p < 0.01$), and the corresponding regression equations were used to predict the minimum concentrations at steady-state in the present study. Thus the concentrations of the glucuronide conjugate were predicted from the equation

$$y = 0.36(x) - 89.51$$

Table 4.4. Simulated minimum steady state plasma concentrations of the glucuronide and sulphate conjugates of paracetamol calculated from the data from a single-dose study of paracetamol 1 g in a group of conservatively managed patients with chronic renal failure (Prescott et al., 1989).

Plasma creatinine ($\mu mol.l^{-1}$)	Predicted glucuronide concentration ($mg.l^{-1}$)	Predicted sulphate concentration ($mg.l^{-1}$)
334	31.4	12.4
378	39.3	26.4
440	59.0	25.7
442	101.6	47.5
484	54.9	29.2
727	241.0	102.1
879	190.0	102.5

where "y" was the expected minimum steady-state concentration of glucuronide conjugate and "x" is the plasma creatinine.

Similarly, for the sulphate conjugate the steady state concentrations were predicted from the following equation:

$$y = 0.18(x) - 44.65$$

where "y" is the expected concentration of the sulphate conjugate at steady state and "x" is the plasma creatinine concentration as before.

Statistics

The statistical significance of differences was determined by the Mann-Whitney test and correlations were determined using standard regression analysis. The concentrations of the conjugates were expressed as paracetamol equivalents.

SECTION 4.3: RESULTS

Paracetamol

The mean daily pretreatment plasma concentrations of paracetamol in the healthy volunteers and patients with chronic renal failure are presented in Table 4.5 and Fig 4.1. Each day the residual plasma concentrations of paracetamol were higher in the renal failure patients than in the volunteers. The mean values over the 10 days of treatment were 3.1 $\pm$0.6 and 1.1 $\pm$0.3 $mg.l^{-1}$ respectively ($p < 0.01$).

Paracetamol glucuronide conjugate

The mean daily pretreatment plasma concentrations of the glucuronide conjugate of paracetamol in the volunteers and patients are presented in Table 4.5 and Fig. 4.2. As expected the mean daily concentrations of the glucuronide conjugate were markedly higher in the patients than in the volunteers.

The mean plasma glucuronide concentrations increased progressively to 87.1 $\pm$67.6 $mg.l^{-1}$ on day 6, after which there was no further accumulation (Table 4.5 and Fig. 4.1). However, there was considerable interindividual variation in the concentrations of retained conjugates at steady-state and in the time taken for this to be reached and the means of the concentrations measured on the 7th to 10th days were therefore taken to represent steady-state values. The mean steady state concentration of the glucuronide conjugate was more than twenty five times higher in the patients than in the volunteers (Table 4.5) and there was a positive correlation with the plasma creatinine concentration ($r=0.97$, $p<0.01$, $n=6$, Figure 4.3).

Paracetamol sulphate conjugate

The mean plasma concentration of the sulphate conjugate in the renal failure patients was 21.7 $\pm$11.2 mg^{-1} after the first 24 h and 24.5 $\pm$18.4 $mg.l^{-1}$ by the 10th day of treatment with little daily variation (Table 4.5 and Fig. 4.4). The steady-state concentrations were over twenty times higher than in the normal volunteers. However, the corresponding correlation between the steady state concentrations of the sulphate conjugate and the plasma creatinine concentration was not statistically significant ($r=0.74$, $p>0.05$, $n=6$, Figure 4.5).

Table 4.5. Mean daily pre-dose plasma concentrations of paracetamol and of its glucuronide and sulphate metabolites ($mg.l^{-1}$) in 6 healthy volunteers and 6 patients with chronic renal failure treated with paracetamol 1 g 3 times a day for 10 days

	Day							
	1	2	3	4	5	6	7-10*	14
Paracetamol concentrations ($mg.l^{-1}$)								
Healthy volunteers	1.3 ±0.5	1.4 ±0.5	1.2 ±0.5	1.2 ±0.7	1.7 ±1.6	1.5 ±0.7	0.8 ±0.5	ND
Renal failure patients	2.4 ±0.4	2.7 ±0.7	3.1 ±1.1	2.9 ±0.9	2.7 ±1.5	3.4 ±2.0	3.1 ±1.3	ND
Glucuronide concentrations ($mg.l^{-1}$)								
Healthy volunteers	2.9 ±0.8	3.8 ±1.3	3.7 ±0.9	3.9 ±1.4	2.6 ±1.5	4.5 ±1.3	3.0 ±0.5	0.3 ±0.6
Renal failure patients	39.1 ±19.7	58.0 ±36.3	68.4 ±47.7	69.9 ±51.3	78.0 ±61.6	87.1 ±67.6	87.0 69.0	4.4 6.6
Sulphate concentrations ($mg.l^{-1}$)								
Healthy volunteers	1.7 ±0.6	1.6 ±0.4	1.4 ±0.6	1.4 ±0.6	1.7 ±1.0	1.9 ±0.9	1.1 ±0.4	0.5 ±0.3
Renal failure patients	21.7 ±11.2	25.1 ±16.6	26.3 ±19.0	24.7 ±17.4	25.0 ±21.1	27.6 ±23.7	25.0 ±19.0	1.4 ±1.9

ND = not detectable
*mean of concentrations on days 7-10

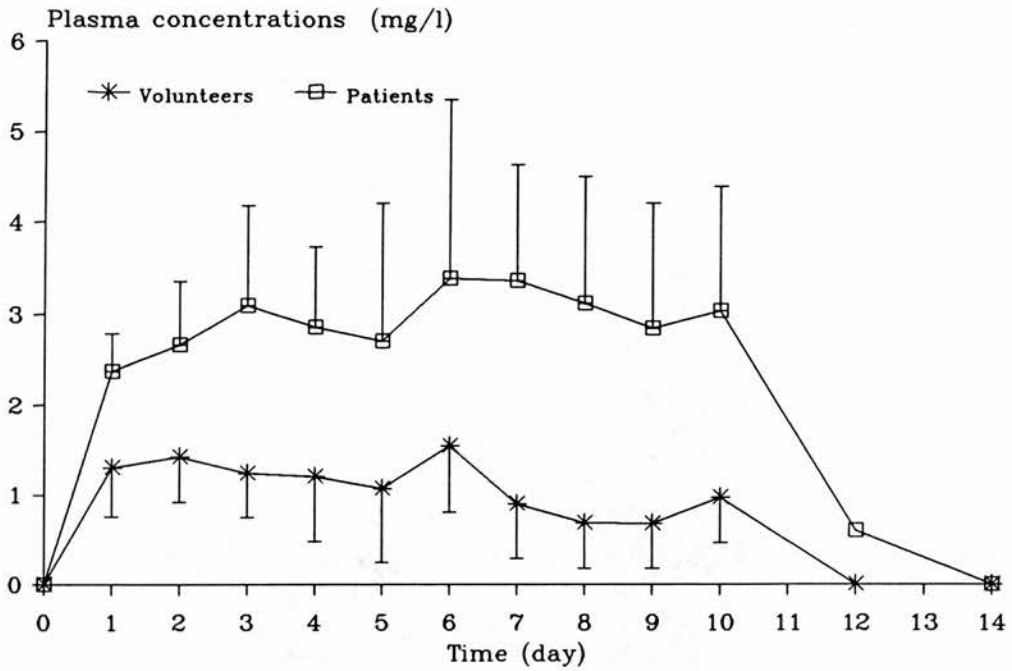


Fig 4.1. Daily pre-dose plasma concentrations (mean ±s.d.) of paracetamol in 6 volunteers and 6 conservatively mananaged patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

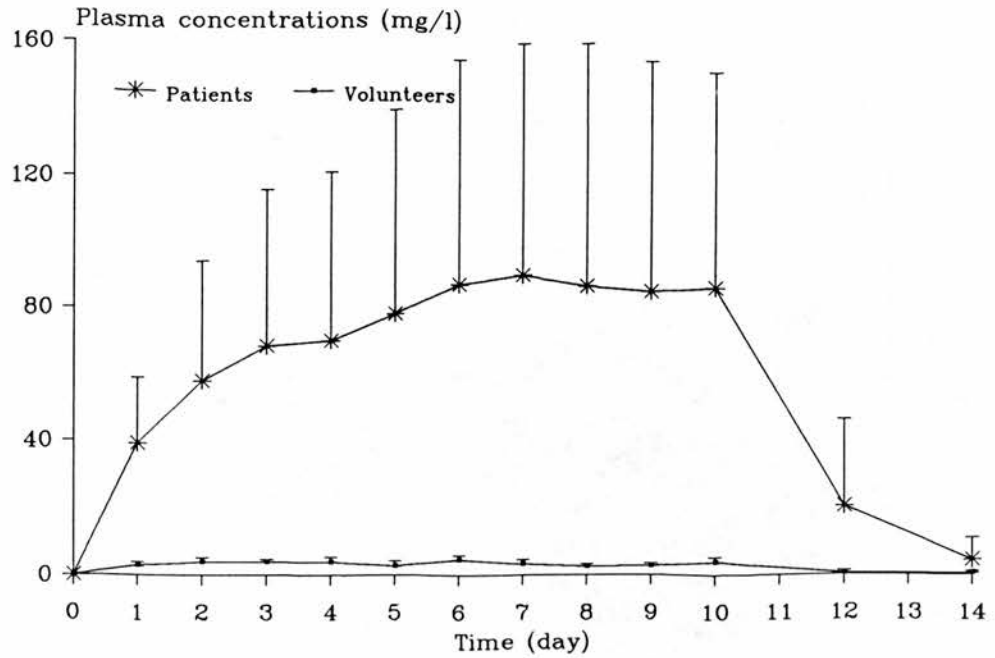


Fig 4.2. Daily pre-dose plasma concentrations (mean ±s.d.) of paracetamol glucuronide in 6 volunteers and 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

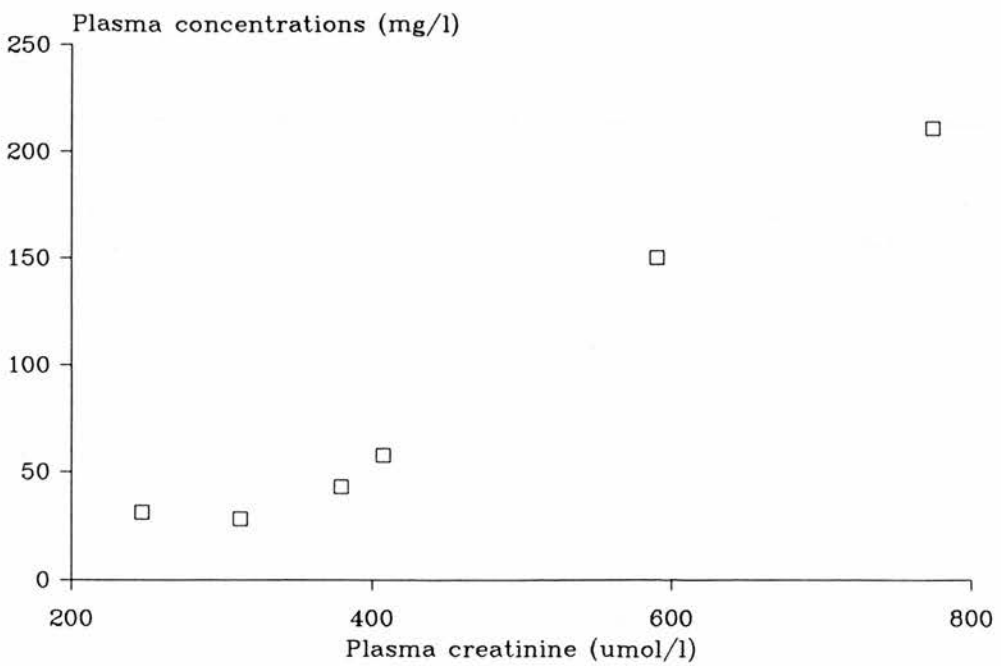


Fig 4.3. Steady-state plasma concentrations of paracetamol glucuronide plotted against plasma creatinine in 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

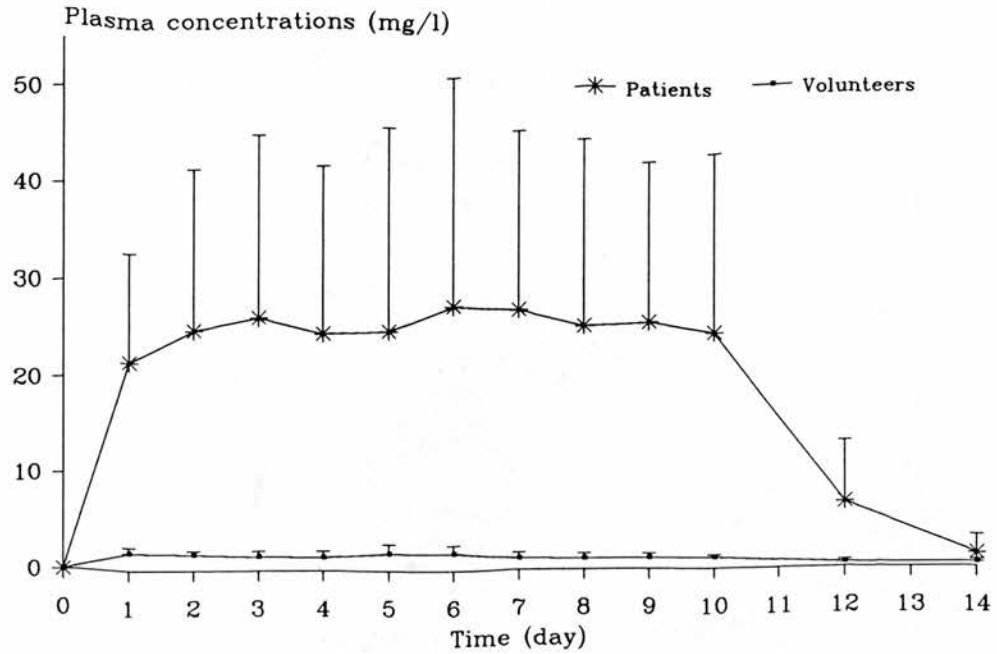


Fig 4.4. Daily pre-dose plasma concentrations (mean ±s.d.) of paracetamol sulphate in 6 volunteers and 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

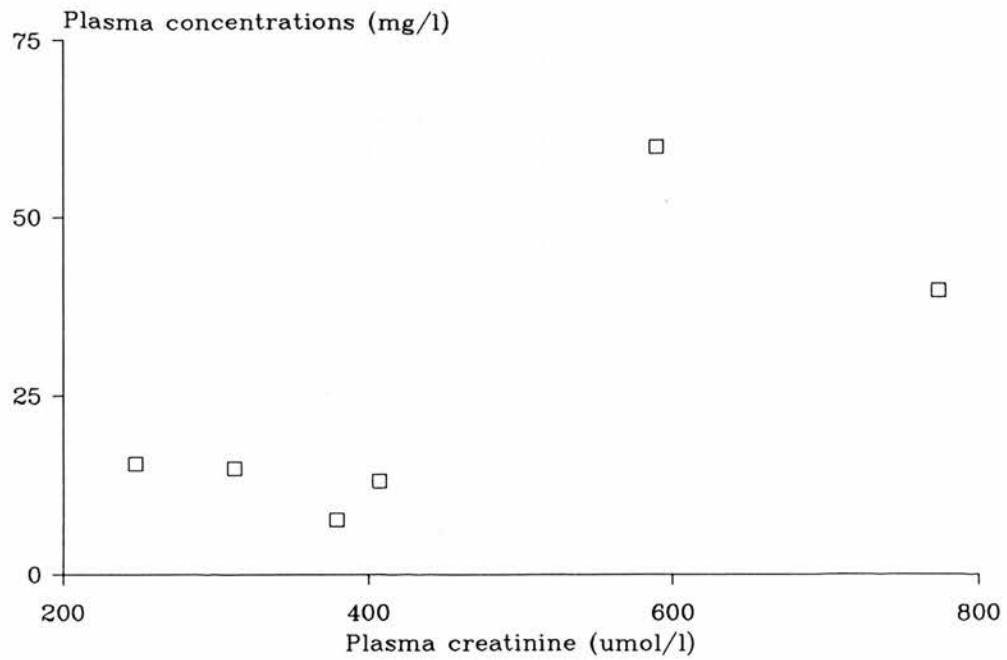


Fig 4.5. Steady-state plasma concentrations of paracetamol sulphate plotted against plasma creatinine in 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

Predicted glucuronide and sulphate conjugate concentrations

The minimum steady-state plasma concentrations of the glucuronide conjugate predicted from the previous single-dose studies were remarkably similar to the concentrations observed at steady-state in the patients with renal failure (Table 4.6, Fig. 4.6) However, predictions of the steady-state concentrations of the sulphate conjugate were accurate in only 3 of the 6 patients, and were less than 50% of those expected in the others (Table 4.6, Fig. 4.7). In the patients in whom the predicted and observed concentrations of sulphate conjugates were similar, the ratios of the plasma concentrations of the glucuronide to sulphate conjugates were less than about 3.0, as expected (2.0, 1.9 and 2.5) whereas in the other three the ratios were much greater (5.7, 4.4 and 5.3).

Cysteine and mercapturate conjugates

The cysteine and mercapturic acid conjugates of paracetamol were detected only in the plasma of the patient with the most severe renal failure and the concentrations were very low.

Plasma concentrations after stopping paracetamol

Paracetamol and its glucuronide and sulphate conjugates disappeared rapidly from the plasma in both volunteers and patients once paracetamol was discontinued (Table 4.5). Only the two patients with the most severe renal failure still had detectable amounts of retained conjugates in the plasma 4 days after stopping the paracetamol.

Table 4.6. The observed and predicted minimum steady state plasma concentrations ($mg.l^{-1}$) of the glucuronide and sulphate conjugate of paracetamol and the ratio of steady state plasma concentrations of the glucuronide and sulphate conjugate in patients (pt) with chronic renal failure taking 1 g paracetamol 3 times a day for 10 days.

Pt. No.	Predicted glucuronide	Observed glucuronide	Predicted sulphate	Observed sulphate	Ratio of observed conjugates
1	58.9	57.7	28.1	13.1	4.4
2	24.3	28.1	11.1	14.8	1.9
3	192.8	209.9	93.7	39.5	5.3
4	48.7	43.2	23.1	7.5	5.7
5	31.4	31.0	12.4	15.5	2.0
6	125.7	150.1	60.8	59.9	2.5

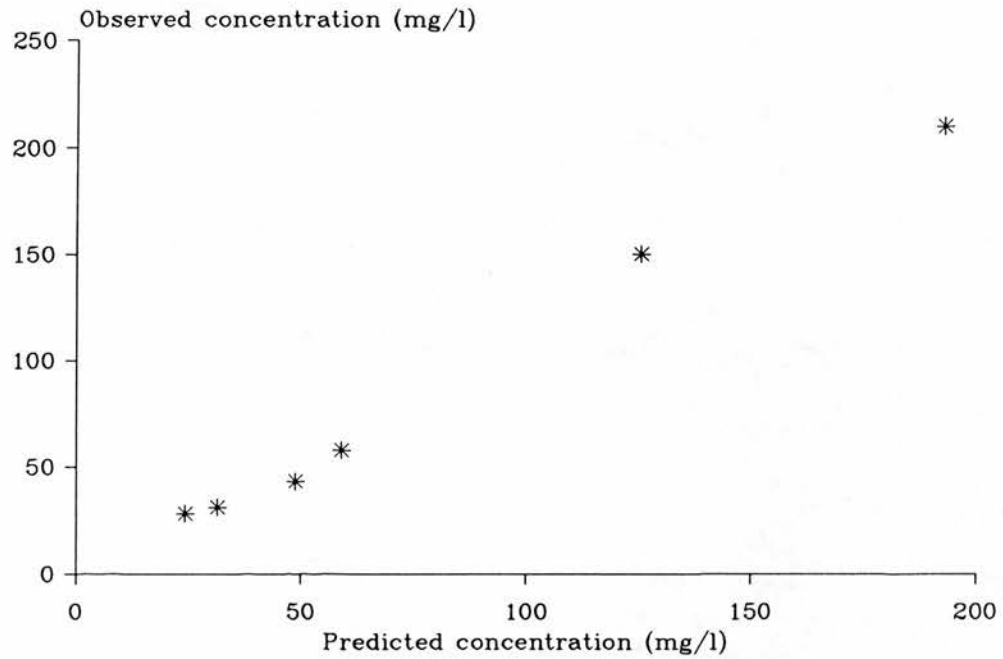


Fig 4.6. Observed and predicted minimum steady-state plasma concentrations of paracetamol glucuronide in 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

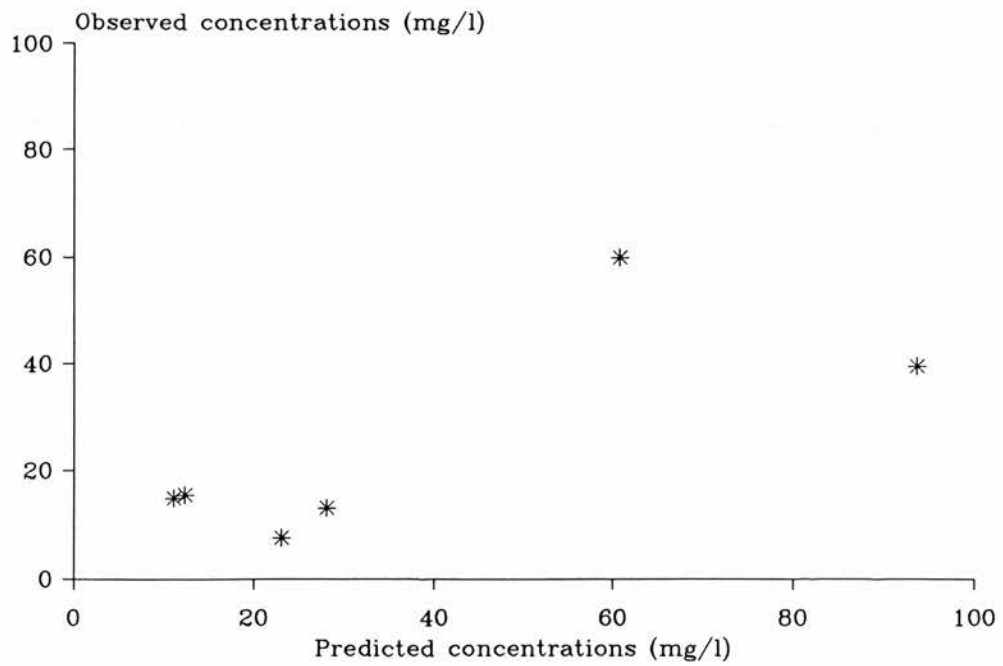


Fig 4.7. Observed and predicted minimum steady-state plasma concentrations of paracetamol sulphate in 6 patients with chronic renal failure taking paracetamol 1 g 3 times a day for 10 days.

SECTION 4.4: DISCUSSION

Oral paracetamol is metabolised extensively by conjugation with glucuronic acid and sulphate. A small fraction of the dose is converted to a reactive intermediate metabolite, which is then conjugated with glutathione and excreted as cysteine and mercapturate conjugates. Over 90% of a therapeutic dose is normally excreted in the urine as metabolites within 24 h (Forrest et al., 1982). In patients with renal failure the ability of the kidney to eliminate polar metabolites is limited, and during repeated dosing significant accumulation of paracetamol conjugates was expected.

When patients with chronic renal failure were given 1 g of paracetamol 3 times daily for 10 days, plasma concentrations of paracetamol were higher than in healthy volunteers taking the same dose. This confirms similar findings in a single-dose study of paracetamol disposition in patients with renal failure (Prescott et al., 1989). The mechanisms are unknown, but it has been suggested by Siegers and Klaassen (1984) that biliary excretion of paracetamol conjugates may become more important when their urinary excretion is reduced in renal failure. They showed that in rats with bilateral ligation of the ureters the amount of paracetamol conjugates cleared by the biliary system was markedly increased. In such circumstances the enterohepatic circulation of glucuronide and sulphate metabolites may be increased and paracetamol may be regenerated by hydrolysis of the conjugates by gastrointesinal flora, with subsequent reabsorption of the parent drug (Verbeeck et al., 1981 (a)).

The mean plasma concentrations of the sulphate and glucuronide conjugates were markedly higher in the patients with renal failure. There was however no evidence that the concentrations of the potentially toxic glutathione-derived conjugates were increased under these circumstances. Steady-state concentrations were reached for the sulphate and glucuronide conjugate by about the 2nd and 6th day respectively. The

plasma concentrations of the glucuronide conjugate at steady-state depended on the severity of the renal failure but this relationship did not hold for the sulphate conjugate. Similarly, the steady-state concentrations of the glucuronide conjugate could be accurately predicted from single-doses studies, but the accumulation of the sulphate conjugate was much less than predicted in 3 of the 6 patients.

Long term or high dose paracetamol therapy is associated with a reduced rate of formation of the sulphate conjugate. This is due primarily to sulphate depletion (Levy & Yamada 1971; Lin & Levy 1981; Clements et al., 1984), although the serum concentrations of inorganic sulphate are not invariably lower than normal (Hendrix-Treacy et al., 1976). A reduced availability of inorganic sulphate may have limited the amount of conjugate formed over the 10 day dosing period in some of our patients. On the other hand, chronic renal failure is often associated with sulphate retention (Freeman & Richards 1979) and so depletion should be less likely to occur even with large doses of paracetamol (Lin & Levy 1982). The patients were taking other medications and it is conceivable that this might have influenced paracetamol metabolism.

Whatever the mechanism, it is clear that the sulphate conjugation of paracetamol was limited in some of the patients with chronic renal failure. Consequently, the concentrations of the glucuronide conjugate were relatively higher, as shown by the increased ratio of glucuronide to the sulphate conjugate formed at steady state. Normally this ratio is about 2:1 (Forrest et al., 1982). This relationship was maintained in the three patients in whom the predicted and observed steady-state concentrations of sulphate conjugate were similar, but not in the other three.

When patients with conservatively managed renal failure took paracetamol regularly for 10 days there was marked accumulation of the glucuronide conjugates as predict-

ed. The results suggested possible enterohepatic cycling of the retained conjugates with regeneration of the parent compound by hydrolysis in the gut, with subsequent reabsorption. Although the sulphate conjugate accumulated over the 10 days it was less than predicted and was not dependent on the degree of renal failure. Further studies of the apparent non-linear sulphate conjugation of paracetamol in patients with renal failure are indicated, using a range of doses, with simultaneous measurement of the serum inorganic sulphate concentrations.

CHAPTER FIVE

THE DISPOSITION OF PARACETAMOL AND ITS CONJUGATES DURING MULTIPLE DOSING IN PATIENTS WITH END STAGE RENAL FAILURE MAINTAINED ON HAEMODIALYSIS

SECTION 5.1: INTRODUCTION

Patients with end-stage renal failure are frequently maintained on haemodialysis. Such patients usually require medication to treat both the renal disease and other related or unrelated conditions (Lee and Marbury, 1984). Due to the marked impairment of renal function, dosage adjustments may be needed for drugs which produce active metabolites or are normally largely excreted unchanged (Bennett et al., 1983). On the other hand a drug may be removed by haemodialysis to the extent that a dosage supplement is required to maintain therapeutic efficacy. Knowledge of the effect of haemodialysis on the elimination of specific drugs is therefore essential for rational dosage regime design in patients undergoing haemodialysis (Lee and Marbury, 1984).

In haemodialysis patients given single doses of paracetamol on an interdialysis day, there was pronounced accumulation of paracetamol glucuronide and sulphate (Lowenthal et al., 1976, Prescott et al., 1989). Furthermore the metabolite concentrations did not fall significantly over 24 h suggesting that haemodialysis was effectively the only method for their elimination (Prescott et al., 1989). It was predicted that the average maximum plasma concentrations of glucuronide and sulphate conjugates would exceed 500 and 400 $mg.l^{-1}$ during regular therapy with 1 g paracetamol 4 times daily in patients with end-stage renal failure on twice weekly maintenance haemodialysis (Prescott et al., 1989).

The object of this study was to establish the disposition of paracetamol and the accumulation of its conjugates in haemodialysis patients given paracetamol regularly over 10 days. In particular, it was hoped to assess the role of haemodialysis in the elimination of retained paracetamol conjugates and to establish whether the procedure compensates adequately for their limited renal excretion in these patients.

SECTION 5.2: METHODS

Patients

Six patients (4 male, 2 female) maintained on haemodialysis for end-stage renal failure (creatinine clearance <5 ml.min^{-1}) were studied. Their mean ages and weights were 53 yr (range 23-63) and 69 kg (range 59-79). Details of their medical histories and medication are presented in Table 5.1 and the results of biochemistry and haematology screening in Table 5.2. Patients were asked to avoid taking paracetamol for 7 days before and for 4 days after the study.

All patients were on maintenance haemodialysis 2 or 3 times weekly as described in Chapter 2. Details of the dialysis regimes are given in Table 5.3.

Experimental design

The patients took paracetamol 1 g (2 x 500 mg soluble "Panadol" tablets) dissolved in approximately 100 ml of water 3 times a day for 10 days (Table 5.4). Food was avoided for 2 h before and for 2 h after dosing. Venous blood was sampled on the first day of the study before starting paracetamol, each day for the next 10 days and on dialysis day in the 2 weeks following the last dose of paracetamol. To avoid disruption of the patients' normal schedules the times of dosing and blood sampling were individualised for each patient depending on whether they were dialysed in the morning or afternoon. Blood was sampled on dialysis days before starting the procedure and on interdialysis days at a set time for each patient (Table 5.4).

In addition, clearance studies were performed on two of the patients (5 and 6) on the day after the last dose (day 10). At the start of dialysis, blood was sampled from the "arterial" and 1 min later from the "venous" side of the extracorporal dialysis circuit (i.e. before and after the dialyser, Chapter 2) and repeated hourly during dialysis.

Table 5.1. Clinical details of six patients with end stage renal failure maintained on haemodialysis who were given 1 g of paracetamol 3 times a day for 10 days.

Patient No.	Age & sex	Medical diagnosis	Oral medications
1	55 F	chronic renal failure ? cause hypertension angina	"Fefol vit" 2 daily glyceryl trinitrate patch nifedepine 10 mg BD temazepam 10 mg nocte calcium resonium 15 g BD
2	62 M	renovascular hypertension atrial fibrillation	"Fefol Vit" 2 daily aluminium hydroxide 950 mg TID digoxin 0.125 mg daily isosorbide mononitrate 20 mg BD nifedepine 10 mg BD labetolol 100 mg BD temazepam 10 mg nocte
3	63 M	rheumatoid arthritis amyloid of kidney hypertension	"Fefol Vit" 2 daily aluminium hydroxide 950 mg TID metoprolol 75 mg daily buprenorphine 400 μg PRN temazepam 10 mg nocte
4	55 F	cystic kidney disease ? type hypertension	"Fefol Vit" 2 daily ferrous sulphate 320 mg mane aluminium hydroxide 475 mg TID metoprolol 50 mg BD 1α cholecalciferol 0.25μg mane calcium resonium 15 g BD
5	23 M	Alport's syndrome	"Fefol Vit" 2 daily ferrous sulphate 320 mg daily aluminium hydroxide 950 mg BD
6	62 M	polycystic kidneys ischaemic heart disease	"Fefol Vit" 2 daily ferrous sulphate 320 mg daily aluminium hydroxide 950 mg TID metoprol 50 mg BD isosorbide mononitrate 10 mg BD diltiazem 60 mg TID 1α cholecalciferol 0.25 μg daily aspirin 75 mg daily

Table 5.2. Clinical biochemical and haematological blood test results in 6 patients with end stage renal failure maintained on haemodialysis taking 1.0 g of paracetamol three times a day for 10 days. Blood was sampled before dialysis.

Plasma concentration	Patients 1	2	3	4	5	6	Normal range
protein $g.l^{-1}$	70	72	68	70	70	69	60-80
albumin $g.l^{-1}$	37	42	37	42	42	43	36-47
calcium $mmol.l^{-1}$	2.8	2.1	2.5	2.4	2.5	2.4	2.1-2.6
phosphate $mmol.l^{-1}$	1.9	2.1	2.0	1.7	1.9	1.7	0.8-1.4
alkaline phosphatase $u.l^{-1}$	83	77	63	74	71	102	40-100
bilirubin $\mu mol.l^{-1}$	3	4	3	4	5	6	2-17
alanine amino-transferase $u.l^{-1}$ (Alt)	16	15	10	14	13	10	10-40
gamma-glutamyl transferase $u.l^{-1}$ (Ggt)	10	11	28	7	6	36	10-55
bicarbonate $mmol.l^{-1}$	22	21	30	17	24	16	24-30
creatinine $\mu mol.l^{-1}$	1166	1407	1094	1263	1604	1094	55-150
urea $mmol.l^{-1}$	28.8	31.8	18.1	23.1	32.9	34.6	2.5-6.6
haemoglobin $g.dl^{-1}$	6.6	7.6	5.1	5.8	10.6	11.1	13-18
white cells $x\ 10^{9}.l^{-1}$	4.9	8.1	4.2	5.1	5.2	5.4	4-11
platelets $x\ 10^{9}.l^{-1}$	292	247	162	201	249	143	150-350

Table 5:3. Details of the dialysis regimes of 6 patients with end stage renal failure on haemodialysis given 1 g paracetamol 3 times a day for 10 days.

Patient No.	Time on dialysis	Dialysis sessions per week	Time of starting dialysis session (h)	Length of session (h)
1	10 months	2	09.30	3.5
2	1 year	2	16.00	4.5
3	3 years	3	09.30	4.0
4	2 years	2	09.30	4.0
5	7 years	3	16.00	5.0
6	10 years	3	16.00	4.0

Table 5.4. Dosing regime and time of blood sampling in 6 patients with end stage renal failure maintained on haemodialysis taking 1 g paracetamol 3 times a day for 10 days.

		Dialysis day		Interdialysis day	
Patient No.	Time of dosing (h)	Time of sampling (h)	Hours since last dose (h)	Time of sampling (h)	Hours since last dose (h)
1	08.00 14.00 22.00	09.30	1.5	18.00	4
2	08.00 14.00 22.00	16.00	2	16.00	2
3	08.00 14.00 22.00	09.30	1.5	19.00	5
4	08.00 14.00 22.00	09.30	1.5	13.00	5
5	08.00 12.00 20.00	16.00	4	16.00	4
6	08.00 12.00 20.00	16.00	4	16.00	4

Samples

All samples were processed as described in Chapter 2.

Drug assay

Plasma concentrations of paracetamol and its conjugates were measured by HPLC as described in Chapter 2.

Pharmacokinetic analysis of data

Two of the patients (5 and 6) had peaks corresponding to paracetamol glucuronide and sulphate on the chromatograms from plasma sampled before starting the study. The presence of the metabolites was confirmed by the addition of ß-glucuronidase and sulphatase as described in Chapter 2. Haemodialysis was carried out before the patients starting taking paracetamol and the remaining samples could not therefore be corrected for the residual amounts of conjugates.

The minimum and maximum steady-state concentrations of the glucuronide and sulphate conjugate which would be expected in haemodialysis patients during regular treatment with paracetamol were calculated using the results of a previous study where single doses of 1 g were given to haemodialysis patients (Prescott et al., 1989 and Prescott LF : personal communication). Predictions based on the superimposition principle were made for patients receiving 1 g paracetamol 4 times a day for 10 days maintained on twice weekly haemodialysis regimes. The clearance of paracetamol glucuronide and sulphate during haemodialysis was assumed to be the same as in an earlier study (Øie et al., 1975). Although the dosing regime was different and 3 of the 6 patients in the present study were on thrice weekly sessions of haemodialysis, the calculations were used as a rough guide to the expected accumulation of the conjugates.

The plasma clearance of paracetamol and its conjugates during haemodialysis was calculated for patients 5 and 6 after every hour of the procedure on the 10th day of the study using Equation 2.9 as described in Chapter 2. This calculates the clearance as the product of the dialyser plasma flow rate and the extraction ratio of paracetamol or its conjugates. The latter is the difference between the "arterial" and "venous" plasma concentrations flowing in and out of the machine divided by the "arterial" concentration. The plasma flow rate is the product of the blood flow rate and (1-haematocrit). During haemodialysis the blood flow rates of patients 5 and 6 were 250 and 200 $ml.min^{-1}$ respectively and these were taken to represent plasma flow rates in the haemodialysis patients because the haematocrits were low due to anaemia (0.33 and 0.28, respectively, normal range 0.4-0.54).

The elimination half life of paracetamol and its conjugates during haemodialysis was estimated from the plasma concentrations of paracetamol taken hourly from the "arterial" side of the fistula during the procedure. The amount of paracetamol and its conjugates removed by dialysis were calculated as:

Amount removed = clearance x AUC Equation 4.1

where clearance = clearance by haemodialysis of paracetamol or its conjugates
AUC = corresponding AUC during haemodialysis of paracetamol or its conjugates

or

Amount removed = clearance x plasma concentration x t Equation 4.2

clearance = clearance calculated each hour, plasma concentration = "arterial" plasma concentration of paracetamol or its conjugates each hour and t = length or dialysis.

The amount of paracetamol or its conjugates present in the body at the start and end of dialysis was calculated as

$$\text{Amount in body} = \text{plasma concentration} \times V_d \qquad \text{Equation 4.3}$$

The total clearance during haemodialysis was calulated as

$$\text{Clearance} = \frac{0.693 \times V_d}{t_{½}} \qquad \text{Equation 4.4}$$

The V_d of paracetamol in patients with renal failure is 0.9 $l.kg^{-1}$ (Forrest et al., 1982) and of its conjugates about 15 l (Lowenthal et al., 1976).

Statistics

The significance of any observed differences between the haemodialysis patients and the CAPD or conservatively managed patients dicussed in Chapters 3 and 4 respectively was determined using the Students t test or Mann Whitney test for un-paired data where appropriate.

SECTION 5.3: RESULTS

Paracetamol

The individual and mean daily plasma concentrations of paracetamol in the 6 patients with the days on which haemodialysis took place are presented in Tables 5.5 and Fig. 5.1.

The mean plasma concentration of paracetamol was 6.8 $\pm$1.9 mg.l^{-1} after the first 24 h of treatment and 6.4 $\pm$2.2 after the 10th day with little daily variation (Table 5.5). Each day blood was sampled either from 1.5 to 2 h or from 4 to 5 h after the last dose depending on the individual patients and whether it was a dialysis or interdialysis day (Table 5.4 and 5.5). Thus, in 5 of the 6 patients sampling was usually carried out 4 or 5 h after the last dose and the concentrations of paracetamol varied from 4 to 11 mg.l^{-1} for each patient (Table 5.5). When single doses of paracetamol 1 g were given to patients with end-stage renal failure maintained on CAPD (Chapter 3) the mean plasma concentration measured 4 to 5 h later was 3.5 $\pm$0.7 mg.l^{-1} . The corresponding concentrations in the haemodialysis patients measured 4 or 5 h after the dose were therefore considerably higher ($p < 0.01$).

Paracetamol glucuronide conjugate

The mean daily plasma concentrations of the glucuronide conjugate of paracetamol are presented in Table 5.6 and Fig 5.1. The mean plasma concentration after the first 24 h of treatment was 46.8 $\pm$9.9 mg.l^{-1} and by the 10th day was 60.3 $\pm$7.3 mg.l^{-1}. Apparent steady-state concentrations of 60.0 $\pm$11.1 mg.l^{-1} were reached by the 2nd day and thereafter concentrations varied little on each subsequent day (Table 5.6). Concentrations measured from the 7th to 10th day were therefore taken to represent steady-state concentrations.

The mean steady-state concentrations of paracetamol glucuronide measured in conservatively managed patients with chronic renal failure treated with a similar regime of paracetamol (Chapter 4) were higher than in the haemodialysis patients at 87.0 $\pm$69.0 compared with 67.5 $\pm$16.4 mg.l^{-1} but the differences failed to reach statistical significance.

Paracetamol sulphate conjugate

The mean daily concentrations of the sulphate conjugate of paracetamol are presented in Table 5.6 and Fig. 5.1. After the first 24 h of treatment the mean plasma concentration of the sulphate conjugate was 39.6 $\pm$15.3 mg.l^{-1} and by the 10th day it was 55.4 $\pm$11.8 mg.l^{-1}. As in the case of the glucuronide conjugate, apparent steady-state concentrations were reached by the 2nd day (54.5 $\pm$10.6 mg.l^{-1}) and thereafter there was little day to day variation (Table 5.5).

The mean steady-state concentration of the sulphate conjugate in the conservatively managed group was 25.0 $\pm$19.0 mg.l^{-1} and this significantly lower than in the haemodialysis group (55.5 $\pm$8.1 mg.l^{-1}, $p<0.01$).

Plasma cysteine and mercapturate conjugates

The mean daily plasma concentrations of the cysteine and mercapturate conjugates of paracetamol are presented in Table 5.6 and Fig. 5.1. All of the patients had measurable amounts of both conjugates but concentrations of the cysteine conjugate were always higher than those of the mercapturic acid conjugate. Mean steady-state concentrations of 5.7 $\pm$2.3 and 3.7 $\pm$1.4 mg.l^{-1} respectively were reached by the 2nd day of treatment and there was little variation thereafter during the rest of the study (Table 5.6).

Table 5.5. Plasma concentrations of paracetamol ($mg.l^{-1}$) in 6 patients with end stage renal failure maintained on haemodialysis given 1 g paracetamol 3 times a day for 10 days. In 5 of the 6 patients blood was mainly sampled 4 or 5 h after the previous dose of paracetamol and the mean of these samples is also presented. Days on which haemodialysis took place are marked with an asterix.

	Day											
Patient No.	1	2	3	4	5	6	7	8	9	10	11-14	Mean of samples taken at 4 or 5 h
1	5.3	3.2	2.3*	3.9	7.7	6.9	N/A*	3.7	6.2	5.7*	0.8	5.3 (n=7)
2	5.3	4.2	5.7	6.6*	8.2	4.1	10.1*	6.3	6.1	9.0	1.1	
3	10.9	9.6	11.7*	12.2	13.0*	8.2	11.7	N/A*	12.6	N/A*	2.0	10.8 (n=5)
4	6.8	11.3	4.6*	13.6	8.8	10.6	6.8*	13.3	11.8	6.3*		10.9 (n=7)
5	5.9*	6.0	8.5*	5.8	6.2*	7.7	5.7	9.3*	4.9	2.3*	1.9	6.7 (n=9)
6	6.6*	3.3	6.8*	3.9	3.4*	3.4	6.6	4.5*	3.2	8.5*		4.6 (n=9)
Mean ±SD	6.8 ±1.9	6.3 ±3.1	6.6 ±3.0	7.7 ±3.9	7.9 ±2.9	6.8 ±2.5	8.2 ±2.3	7.4 ±3.5	7.5 ±3.5	6.4 ±2.2	1.5 0.5	

N/A=not available

Table 5.6. Mean daily plasma concentrations of the glucuronide, sulphate, cysteine and mercapturic acid conjugates of paracetamol ($mg.l^{-1}$) in 6 patients with end stage renal failure maintained on haemodialysis treated with paracetamol 1 g 3 times a day for 10 days.

Day											
1	2	3	4	5	6	7	8	9	10	11-14	17-21
Glucuronide concentrations											
46.8 ± 9.9	60.0 ±11.1	61.9 ±13.6	59.9 ±15.4	64.2 ±18.0	61.9 ±16.2	70.0 ±16.5	58.8 ± 5.6	66.5 ±15.9	60.3 ± 7.3	54.3 ±10.3	13.7 ± 8.1
Sulphate concentrations											
39.6 ±15.3	54.5 ±10.6	58.2 ±10.4	50.8 ±13.1	54.6 ± 9.5	52.0 ± 9.8	59.6 ± 3.1	50.0 ± 9.9	53.3 ± 8.9	55.4 ±11.8	19.3 ± 6.6	6.4 ±2.1
Cysteine concentrations											
4.2 ±1.7	5.7 ±2.3	6.4 ±2.0	5.7 ±2.2	6.9 ±3.1	6.5 ±2.7	7.1 ±2.2	6.9 ±2.5	7.5 ±2.9	6.7 ±3.6	1.8 ±0.8	0.9 ±1.3
Mercapturate concentrations											
2.4 ±0.8	3.7 ±1.4	4.1 ±1.2	3.3 ±1.3	3.9 ±1.6	3.6 ±1.6	4.4 ±1.8	3.3 ±0.6	4.1 ±1.7	3.7 ±1.2	1.7 ±0.4	ND ND

ND=not detectable

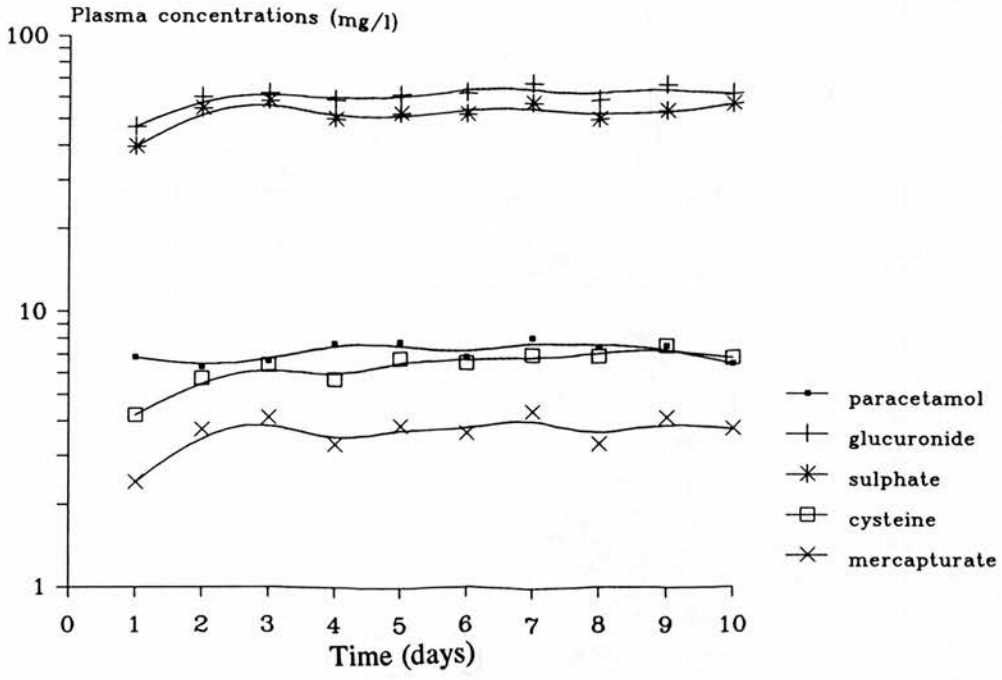


Fig 5.1. Mean plasma concentrations of paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates in 6 patients with end stage renal failure maintained on haemodialysis taking 1 g paracetamol 3 times a day for 10 days.

Predicted glucuronide and sulphate conjugates concentrations

Based on the results of a previous single dose study the minimum and maximum plasma concentrations of the glucuronide conjugates which would be expected in haemodialysis patients taking 1 g paracetamol 4 times a day for 10 days with twice weekly haemodialysis sessions were 101 $\pm$37 and 569 $\pm$150 $mg.l^{-1}$ respectively (Prescott et al., 1989 and Prescott : personal communication). The corresponding concentrations expected for the sulphate conjugate were 123 $\pm$24 and 434 $\pm$92 $mg.l^{-1}$ respectively.

In the present study most of the blood sampling took place either 4 or 5 h after the last dose of paracetamol (Table 5.4). In patients with end-stage renal failure maintained on CAPD given single doses of paracetamol it took about 5 h to reach maximum plasma concentrations of the glucuronide and sulphate conjugate (Chapter 3). Therefore, in the present study the plasma concentrations of the glucuronide and sulphate conjugates were measured when maximum or near maximum concentrations would be expected. The observed mean steady-state concentrations of the glucuronide and sulphate conjugate of 67.5 $\pm$16.4 and 55.5 $\pm$8.1 $mg.l^{-1}$ respectively were therefore much lower than the predicted maximum concentrations of 569 $\pm$150 and 434 $\pm$92 $mg.l^{-1}$ respectively. Indeed the observed near maximum concentrations were much less than the predicted minimum steady-state concentrations shown above. Furthermore the ratio of the steady-state concentrations of the glucuronide and sulphate conjugate was 1:1 apart from patient 3 where a slightly higher ratio of 1.8:1 was found.

Clearance of paracetamol and its conjugates during haemodialysis

The hourly concentrations of paracetamol and its glucuronide, sulphate, cysteine and mercapturate conjugates in blood entering and leaving the dialyser, extraction ratios and clearances by haemodialysis in patients 5 and 6 are presented in Table 5.7 and 5.8 and results are presented graphically in Fig. 5.2 and 5.3.

Table 5.7. Plasma concentrations of paracetamol and its glucuronide and sulphate conjugates sampled from the "arterial" and "venous" sides of the of the extracorporal dialysis circuit (i.e. before and after the dialyser) during haemodialysis on day 10 in 2 patients with end stage renal failure given 1 g paracetamol 3 times a day for 10 days. Extraction ratios and clearances during haemodialysis were calculated as described in Chapters 2 & 5.

Patient No.	Time (h)	Concentration Arterial ($mg.l^{-1}$)	Venous	Extraction ratio	Clearance ($ml.min^{-1}$)
		Paracetamol			
5	1	2.2	1.3	0.40	98.8
6	1	3.5	2.5	0.29	57.1
5	2	2.1	1.2	0.41	102.2
6	2	2.9	2.0	0.32	63.9
5	3	1.7	1.1	0.37	91.9
6	3	2.4	1.3	0.44	88.2
5	4	1.6	1.0	0.36	90.5
6	4	1.7	1.2	0.27	54.1
5	5	1.5	0.6	0.59	149.6
		Glucuronide			
5	1	47.3	41.5	0.12	30.3
6	1	62.1	55.2	0.11	22.2
5	2	47.7	44.9	0.06	14.8
6	2	56.6	50.1	0.11	22.9
5	3	48.5	42.4	0.12	31.2
6	3	56.8	48.2	0.15	30.3
5	4	44.4	38.7	0.13	31.8
6	4	57.6	44.8	0.22	44.5
5	5	45.5	33.1	0.27	68.2
		Sulphate			
5	1	44.6	26.7	0.40	122.5
6	1	41.3	25.6	0.38	71.3
5	2	39.7	30.2	0.24	128.6
6	2	34.8	20.1	0.42	85.9
5	3	35.5	17.6	0.50	126.9
6	3	30.2	17.5	0.42	104.9
5	4	27.7	14.9	0.46	140.8
6	4	26.8	12.3	0.54	68.1
5	5	24.5	8.4	0.66	189.7

Table 5.8. Plasma concentrations of the cysteine and mercapturate conjugates of paracetamol sampled from the arterial and venous lines of the extracorporal dialysis circuit (i.e. before and after the dialyser) during haemodialysis on day 10 in 2 patients with end stage renal failure given 1 g paracetamol 3 times a day for 10 days. Extraction ratios and clearances during haemodialysis were calculated as described in Chapters 2 and 5.

Patient No.	Time (h)	Concentration Arterial ($mg.l^{-1}$)	Concentration Venous ($mg.l^{-1}$)	Extraction ratio	Clearance ($ml.min^{-1}$)
		Cysteine			
5	1	2.6	1.3	0.49	122.6
6	1	2.6	1.4	0.44	88.4
5	2	2.3	1.1	0.52	128.8
6	2	2.4	1.1	0.51	102.98
5	3	1.9	0.9	0.55	137.4
6	3	2.0	1.1	0.46	91.8
5	4	1.7	0.8	0.56	141.0
6	4	1.8	1.0	0.43	86.5
5	5	1.3	0.4	0.73	181.3
		Mercapturate			
5	1	2.0	1.0	0.49	122.5
6	1	1.6	1.0	0.36	71.3
5	2	1.7	0.8	0.51	128.7
6	2	1.2	0.7	0.43	86.0
5	3	1.3	0.6	0.51	126.9
6	3	1.0	0.5	0.52	104.9
5	4	1.2	0.5	0.56	140.8
6	4	0.9	0.6	0.34	68.1
5	5	0.9	0.2	0.76	189.7

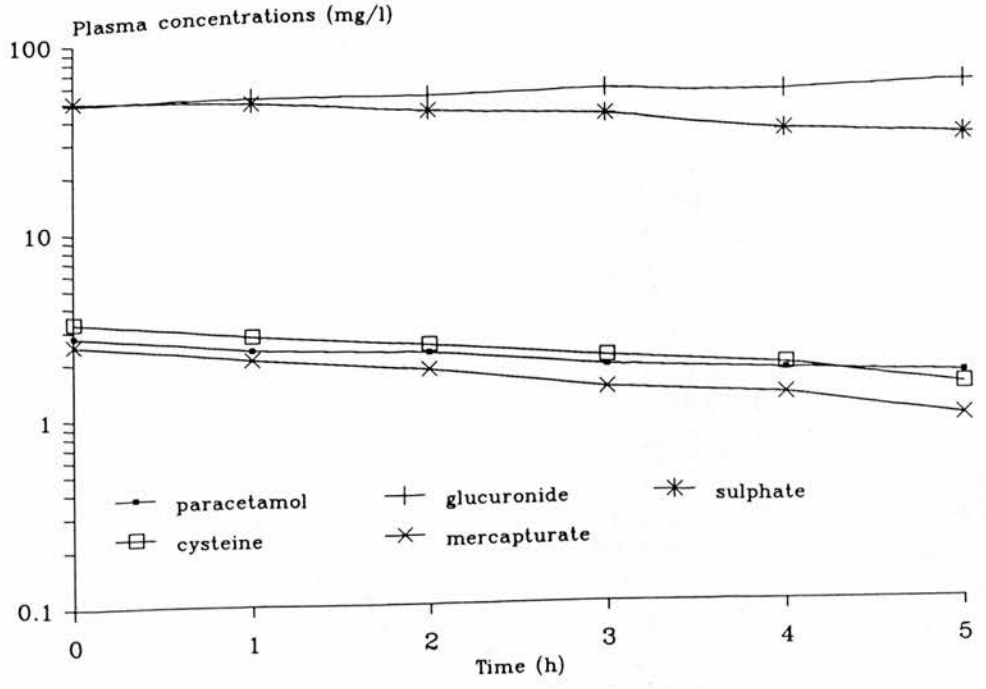


Fig 5.2. "Arterial" plasma concentrations of paracetamol and its glucuronide, sulphate, cysteine and mercapturic acid conjugates measured during haemodialysis in a patient (patient 5) with end stage renal failure taking 1 g paracetamol 3 times a day for 10 days.

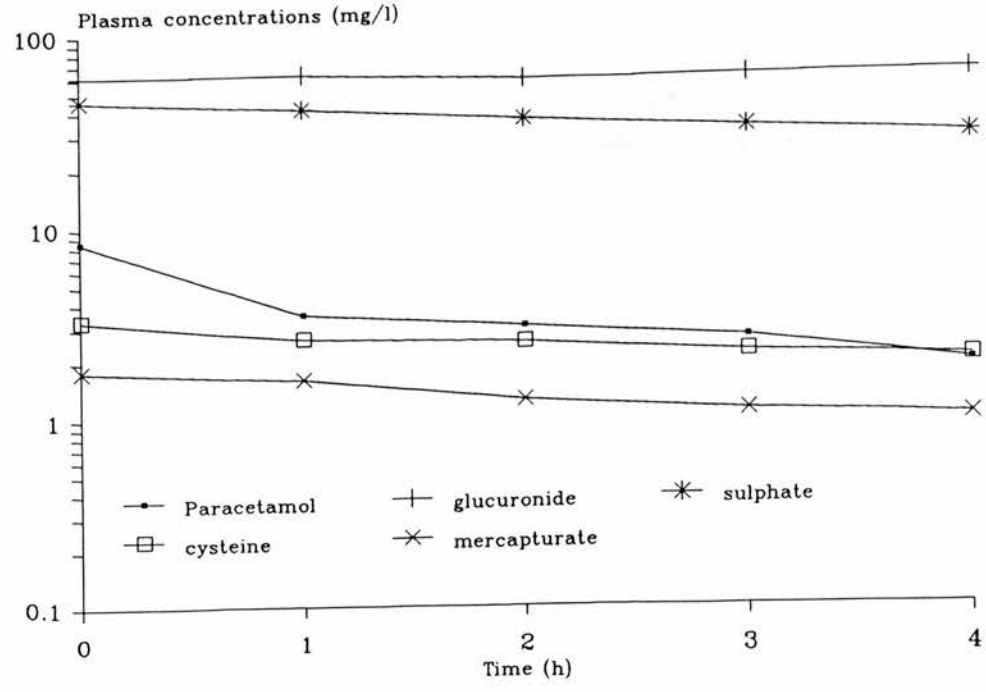


Fig 5.3. The corresponding concentrations in patient 6.

The mean extraction ratios of paracetamol in patients 5 and 6 were 42.4% and 26.3% respectively and the corresponding mean clearances were 106.0 and 65.8 $ml.min^{-1}$ (Table 5.7). Similar values were noted for the sulphate conjugate (Table 5.7) but the extraction ratios and plasma clearances for the glucuronide conjugate were much lower in both patients (14.1 and 12.0% and 35.3 and 24.0 $ml.min^{-1}$ for patients 5 and 6 respectively, Table 5.7).

The extraction ratios of the cysteine and mercapturate conjugate were similar to those calculated for paracetamol and its sulphate conjugate as were the plasma clearances during haemodialysis although the latter were slightly higher in patient 5 at 140 $ml.min^{-1}$ for both conjugates (Table 5.8).

Elimination half life of paracetamol and its conjugates during haemodialysis

The elimination half life of paracetamol and its conjugates in patients 5 and 6 are presented in Table 5.9. During haemodialysis the $t_{½}$ of paracetamol was 5.6 and 2.9 h in patients 5 and 6 respectively and values for the sulphate, cysteine and mercapturic acid conjugates ranged from 3 to 5 hours (Table 5.9). The $t_{½}$ of the glucuronide conjugate during dialysis was markedly prolonged, however, at 46.4 and 31.9 h in patients 5 and 6 respectively and this is in keeping with the limited clearance of the conjugate during the procedure.

Amount of paracetamol and its conjugates removed during haemodialysis

The amount of paracetamol and its conjugates removed during dialysis and the amounts present in the body at the beginning and end of the procedure are presented in Table 5.9. At the beginning of the procedure 195 and 597 mg of paracetamol were present in patients 5 and 6 respectively. The amounts of the glucuronide (731 and 921 mg respectively) and sulphate conjugate were similar in each patient (751 mg and 688 mg respectively). The concentations of the glucuronide conjugate were therefore

equivalent to only 1 dose of 1 g paracetamol and the sulphate to 2 doses if paracetamol was metabolised to its glucuronide and sulphate conjugate in the normal ratio of 2:1.

There was good agreement using Equations 4.1 and 4.2 to calculate the amounts of paracetamol and its conjugates removed during haemodialysis. Similar amounts of paracetamol and its cysteine and mercapturate conjugates were removed (Table 5.9). Despite the fact that there were similar amounts of paracetamol glucuronide and sulphate present in the body at the start of the procedure, much less glucuronide was removed than sulphate during haemodialysis (494 and 423 versus 1573 and 700 mg, Table 5.9).

When the difference between the amount of paracetamol and its conjugates present in the body at the start and end of dialysis was calculated considerably more paracetamol had been removed during dialysis than estimated from the dialysis clearances (Table 5.9). This presumably reflects clearance by metabolism as well as dialysis. On the other hand, the amounts of the glucuronide and sulphate metabolites removed during haemodialysis appeared to be much less when calculated by this method but this may also reflect their ongoing production from paracetamol metabolism. In agreement with this the total clearance of paracetamol in both patients calculated from Equation 4.4 was much greater (144 and 297 $ml.min^{-1}$) than the corresponding clearances by haemodialysis calculated from the extraction ratios (106.0 and 52.7 $ml.min^{-1}$) and the clearances of the glucuronide and sulphate conjugates was much less (Table 5.9).

Table 5.9. Elimination half life ($t_{½}$) of paracetamol and its conjugates, plasma clearance (0.693 x $V_d/t_{½}$), amount removed from the body during haemodialysis (HD) (clearance x AUC during dialysis) or (mean hourly clearance x hourly "arterial" plasma concentrations (c) x time (t) of dialysis), where clearance is calculted from the extraction ratio of the dialyser, the amount present in the body at the start and end of the procedure (plasma concentration x V_d) and the calculated differences in amounts in 2 haemodialysis patients taking 1 g paracetamol 3 times a day for 10 days. Calculations were performed during haemodialysis on the tenth day of the study.

	$t_{½}$	Clear-ance	Amount removed during HD		Amount in body during HD		
	(h)	($ml.min^{-1}$)	Cl x AUC (mg)	Cl x c x t (mg)	start (mg)	end (mg)	Start-end (mg)
Paracetamol	5.6	144.4	62	57	195	42	153
	2.9	278.8	54	41	597	84	513
Glucuronide conjugate	46.4	3.7	494	480	731	496	235
	31.9	5.4	423	408	921	672	279
Sulphate conjugate	4.7	36.9	1572	1410	751	126	625
	5.0	34.7	700	648	688	185	503
Cysteine conjugate	4.0		92	81			
	4.6		53	50			
Mercapturate conjugate	3.5		67	57			
	3.3		26	21			

Plasma concentrations after stopping paracetamol

Plasma concentrations of paracetamol and its conjugates were measured in 5 of the 6 patients within 4 days of stopping paracetamol (Tables 5.5 and 5.6). All of the patients had been dialysed on day 10 and these samples were taken from days 11 to 14 before any further haemodialysis had taken place. Patient 6 had large concentrations of paracetamol in his plasma suggesting recent ingestion so the data were omitted. All of the other 4 patients had measurable quantities of paracetamol in the plasma and the mean concentration was 1.5 $\pm$0.5 mg.l^{-1}. The corresponding mean concentration of the glucuronide conjugate was 54.3 $\pm$10.3 mg.l^{-1} which was similar to that observed during treatment (Table 5.6). The concentration of the sulphate conjugate was lower than during the treatment days at 19.3 $\pm$6.6 mg.l^{-1} as were concentrations of the cysteine and mercapturate conjugates at 1.8 $\pm$0.8 and 1.7 $\pm$0.4 respectively (Table 5.6).

Further samples were taken in 4 of the 6 patients between 7 and 10 days of stopping treatment. Small amounts of paracetamol were present in 3 of the 4 patients with mean concentrations of 0.6 $\pm$0.4 mg.l^{-1} (Table 5.5). The glucuronide conjugate was present in the plasma at mean concentrations of 13.7 $\pm$8.1 mg.l^{-1} and the sulphate conjugate at 6.4 $\pm$2.1 mg.l^{-1}. The cysteine but not the mercapturate conjugate was still measurable in 2 patients (Table 5.6).

SECTION 5.4: DISCUSSION

In haemodialysis patients taking regular doses of paracetamol the concentrations of paracetamol measured 4 or 5 h after the last dose were higher than expected based on the results of the single dose study in a similar group of patients with end-stage renal failure maintained on CAPD (Chapter 3). This confirms the findings in conservatively managed patients with chronic renal failure on a similar regime of paracetamol where higher than normal concentrations of paracetamol were measured in the plasma several hours after the previous dose (Chapter 4).

Although the results may be explained by enterohepatic recycling of retained paracetamol conjugates with regeneration and subsequent reabsorption of the parent compound (Verbeeck et al., 1981 (a)) an alternative explanation is limited conjugation to paracetamol glucuronide and sulphate under conditions of prolonged dosing (Slattery and Levy, 1979). In keeping with this, the accumulation of the glucuronide and sulphate metabolite was much less than had been predicted from a previous single dose study in haemodialysis patients and the elimination half life of paracetamol was prolonged in one of the 2 patients in whom it was measured.

The limited capacity of the body to metabolise paracetamol to its sulphate conjugate has been well documented (Galinsky and Levy, 1981, Clements et al., 1984, Lin and Levy, 1981, Hendrix-Treacy et al., 1986) and is thought to be due to the limited availability and consequent depletion of inorganic sulphate. Infusion of paracetamol in rats was associated with pronounced accumulation of paracetamol in the plasma due to decreased formation of the sulphate metabolite which could be prevented by concomitant infusion of sodium sulphate (Galinsky and Levy, 1981).

Paradoxically, sulphate retention occurs in renal failure and is thought to be a conse-

quence of decreased renal excretion of sulphate together with continued ingestion of sulphur containing foodstuffs (Freeman and Richards, 1979). Although haemodialysis has been shown to significantly reduce the plasma sulphate in patients with end-stage renal failure (Holmes et al., 1960) the amount removed may depend on the type of system used for haemodialysis (Freeman and Richards, 1979). Even when a distinct decrement in serum sulphate occured during haemodialysis the post dialysis values were still elevated compared to normal and the subsequent predialysis sulphate values were 4 to 5 times greater than in normal subjects.

The conjugation of paracetamol with sulphate is facilitated in animals with renal failure due to the retention of inorganic sulphate and sulphate depletion does not occur even during prolonged intravenous infusion of paracetamol (Lin and Levy, 1982). It is thought, however, that depletion would occur eventually if the administration rate of paracetamol was sufficiently high and prolonged (Levy, 1986).

This may explain why the sulphate conjugate did not accumulate as predicted in the haemodialysis patients during the 10 days of paracetamol treatment despite the fact that sulphate retention was likely. On the other hand it may explain the altered ratio of paracetamol glucuronide to sulphate in the plasma which indicated that concentrations of the conjugates were present in roughly equal proportions rather than the 2:1 ratio normally expected. The accumulation of the sulphate conjugate was significantly less in the conservatively managed patients possibly because sulphate retention would be less marked with that degree of renal impairment. Furthermore, conservatively managed patients are restricted in their intake of protein including the sulphur containing amino acids whereas haemodialysis are allowed to eat more protein presumably leading to higher concentrations of inorganic sulphate available for conjugation.

Following a single dose of paracetamol in patients with chronic renal failure the frac-

tional urinary recovery of paracetamol glucuronide was normal (Prescott et al., 1989). There is some evidence, however, that glucuronide conjugation may also be limited in man (Slattery and Levy, 1979). Although this has been demonstrated for salicylamide (Levy and Matsuzawa, 1967) and salicylic acid (Levy et al., 1972) it has not been feasible to administer sufficiently large doses of paracetamol to human subjects to test the hypothesis but analysis of urine samples following self administration in suicide attempts indicate that the fractional conversion of the drug to both its sulphate and glucuronide conjugate decreased with increasing dose (Slattery and Levy, 1979). Furthermore, inhibition of paracetamol sulphate formation by concomitant administration of ascorbic acid causes an increase in the fraction of paracetamol excreted unchanged and as the glucuronide conjugate but the latter was not increased as much as expected (Houston and Levy, 1976). Increasing doses of paracetamol in rats were associated with a less than proportional increase in the rate of formation of paracetamol glucuronide (Galinsky and Levy, 1979).

The evidence for saturation of glucuronide metabolism in overdose patients has been challenged however and results from other studies in patients without liver damage following paracetamol overdose show an increased ratio of paracetamol glucuronide to the parent compound and the sulphate conjugate (Prescott, 1983). Although glucuronide conjugation becomes impaired in the presence of hepatic necrosis following overdose there was no evidence of saturation kinetics of the pathway in patients without liver damage based on a number of studies (Prescott, 1983).

Another explanation for the lower than predicted plasma concentrations of the glucuronide conjugate might be increased biliary excretion of the retained metabolites in the haemodialysis patients. Enterohepatic recycling may become more important in renal failure and increased biliary excretion of the glucuronide conjugate with hydrolysis in the gut and subsequent reabsorption of the parent compound paracetamol may have

compensated for the reduced renal clearance in these patients (Verbeeck et al.,1981 and Siegers and Klaassen, 1984). In addition, various tissues in the body may possess glucuronidase activity which may lead to deconjugation and liberation of the paracetamol (Wakabayashi, 1970). The patients were all taking a variety of other medications which could have conceivably altered the metabolism of paracetamol.

If glucuronide (or sulphate) metabolism were limited in these patients then increased formation of the glutathione-derived conjugatesmight be expected. In healthy subjects given 1 g paracetamol the ratios of paracetamol and its glucuronide, sulphate, cysteine and mercapturate conjugates in the urine were 4.1 : 60.7 : 28.1 : 3.0 and 4.1 % and similar ratios were seen in patients with impaired renal function given the same dose (Prescott et al., 1989). In patients who had taken paracetamol overdoses but had not suffered liver damage the corresponding ratios were 8.6 : 75.3 : 9.3 : 6.9 % where the last value refers to combined cysteine and mercapturate conjugates. The latter value was increased to 15.2 % in patients who had suffered liver damage (Prescott, 1983). In the present study the ratios of steady-state plasma concentrations (from day 7 to 10) of paracetamol and its conjugates were 7.7: 67.5 : 55.5 : 6.7 : 4.1 $mg.l^{-1}$. The combined cysteine and mercapturate conjugates were therefore in an appropriate ratio to the paracetamol and paracetamol glucuronide suggesting that production of the glutathione-derived conjugates was not increased in the haemodialysis patients.

In patients with end-stage renal failure, extraction ratios of 0.46 to 0.78 for paracetamol and 0.13 to 0.60 for paracetamol sulphate during haemodialysis were reported and these are in agreement with the present findings (Oie et al., 1976). Similarly, in another study, the extraction efficiency of dialysis averaged 47.5 and 43 % for the parent compound and total metabolites (glucuronide and sulphate) respectively (Lee et al., 1981). The corresponding dialysis clearances were 112 $ml.min^{-1}$ and 105.8 $ml.min^{-1}$ respectively.

The extraction ratio of the glucuronide metabolite in the present study was surprisingly low in the 2 subjects and the corresponding clearance during dialysis was less than 40 $ml.min^{-1}$ in both cases. In addition, much less of the glucuronide than sulphate conjugate was removed during dialysis despite similar amounts of both being present in the body at the start of the procedure and the elimination half life of the former conjugate was greatly prolonged.

It is unclear why less paracetamol glucuronide was cleared during haemodialysis and the results should be interpreted cautiously because data was available from only 2 patients. The low clearance may possibly be due to the greater size of the glucuronide compared to the sulphate conjugate and presumably explains why the plasma concentrations of the glucuronide conjugate fell less rapidly once paracetamol was stopped.

Despite limited clearance by haemodialysis, a concentration of the glucuronide conjugate equivalent to only 1 g paracetamol was present in the body at the start of the procedure on the 10th day of the study. Possible explanations for this finding include reduced absorption of paracetamol or reduced metabolism to the glucuronide conjugate, both of which seem unlikely, as has been discussed or increased biliary excretion of the retained conjugates under these conditions.

In these patients with end-stage renal failure maintained on haemodialysis the accumulation of the glucuronide and sulphate conjugates of paracetamol during 10 days therapy was much less than had been predicted from a single dose study. Relatively more of the sulphate conjugate was formed in relation to the glucuronide conjugate, however, possibly due to sulphate retention in these patients. There was some evidence of enterohepatic recycling of the retained conjugates and higher than expected concentrations of the parent compound was measured each day in the plasma. Satura-

tion of the glucuronide and sulphate pathways also may have occurred during chronic therapy but stauration of the former metabolic pathway is exceedingly unlikely. Clearance of the glucuronide conjugate by haemodialysis appeared to be restricted. There was no evidence of increased production of the potentially toxic glutathione-derived conjugates under the conditions of the study.

Further studies are indicated to establish the capacity of these patients to conjugate paracetamol during chronic dosing and to establish associated changes in serum sulphate concentrations. In addition more studies are needed to establish whether the clearance of paracetamol glucuronide is limited during haemodialysis which might have implications for the clearance of other drug metabolites during the procedure.

CHAPTER 6

STUDIES WITH SINGLE DOSES OF FRUSEMIDE IN HEALTHY MALE VOLUNTEERS

SECTION 6.1: INTRODUCTION

The potent loop diuretic frusemide is used in the treatment of oedematous states associated with cardiac, hepatic and renal failure. Therapy is frequently complicated by apparently erratic systemic availability from the oral route and from unpredictable responses to a given dose (Boles-Ponto and Schoenwald, 1990). Frusemide is believed to act at the surface of the ascending limb of the loop of Henle by inhibiting the active reabsorption of chloride (Burg, 1973). It is delivered to its site of action by active secretion via the non-specific organic acid pump in the proximal tubule and the response is related to the concentration of the drug in the urine rather than in the plasma (Chennavasin et al., 1979).

The pharmacokinetic behaviour of frusemide is characterised by a large degree of variability (Benet, 1979). Although this may be in part due to differences between subjects, study protocols or the assays used to estimate frusemide, a large proportion appears to be due to inter- and intra-subject variation. In addition, various disease states such as renal impairment will alter its disposition. This is particularly important in view of the fact that the fraction of the dose excreted in the urine represents the amount available for pharmacological action.

The object of this study was to investigate the disposition of frusemide in a group of healthy drug-free male volunteers with normal renal function. They received single doses of frusemide both orally and intravenously with concurrent rehydration and serial measurements of frusemide concentrations in the plasma and urine were performed. It was thus hoped to characterise the handling of frusemide by healthy subjects before attempting to elucidate its disposition in patients with chronic renal impairment.

SECTION 6.2: METHODS

Volunteers

Eight healthy male volunteers of mean age 38 yr (range 29 - 54) and weight 89 kg (range 79 - 98) were studied. They had no medical illness, physical examination was normal, they were on no medication, they did not smoke and they all claimed to drink less than 5 units of alcohol per week. The results of routine biochemical and haematological screening tests are presented in Table 6.1.

Experimental design

Each subject was studied on 2 occasions. On the first, the fasting volunteers attended at the Clinical Pharmacology Unit at 08.30 h and a cannula was inserted into a forearm vein. Frusemide tablets 40 mg (Hoechst U.K. Ltd.) were administered orally with 100 ml of water and the volunteers remained recumbent for one hour. 100 ml of water was given at 15 min intervals during this first hour and thereafter fluid was replaced according to the volume of urine passed at the 2 hourly collection times. Breakfast (toast and water or still orange juice) was served one hour after the administration of frusemide (09.30 h) and lunch was taken at 13.00 h.

Blood was sampled before and at 7.5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after the frusemide was given and urine was collected at 0, 30, 60, 120, 240, 360 and 480 min. Subjects were free to go at 16.30 h and were asked to collect all urine passed until the end of the 24 hour period after the administration of frusemide.

On the second study day the procedure was the same but frusemide 40 mg was infused intravenously over one hour at a constant rate in 400 ml of 0.9% saline via an "IMED 960" volumetric infusion pump (Warner-Lambert Health Tech. Ltd., England). Extra

Table 6.1: Results of routine biochemistry and haematology screening in 8 healthy volunteers give 40 mg frusemide orally and by intravenous infusion over 1 h.

	Volunteer								
Plasma concentration	1	2	3	4	5	6	7	8	Normal range
protein $g.l^{-1}$	78	81	72	72	73	71	70	72	60-80
albumin $g.l^{-1}$	49	46	46	44	45	46	45	46	36-47
alkaline phosphatase $u.l^{-1}$	91	70	72	84	90	70	80	68	40-100
bilirubin $\mu mol.l^{-1}$	15	8	10	8	16	9	10	11	2-17
alanine amino-transferase $u.l^{-1}$ (Alt)	31	28	38	68	16	18	20	31	10-40
gamma-glutamyl transferase $u.l^{-1}$ (GGT)	31	12	35	55	16	18	20	23	10-55
bicarbonate $mmol.l^{-1}$	31	24	26	21	30	22	23	25	24-30
creatinine $\mu mol.l^{-1}$	91	99	93	92	99	91	99	98	55-150
urea $mmol.l^{-1}$	4.7	3.9	4.8	4.6	7.0	6.8	3.1	3.6	2.5-6.6
calcium $mmol.l^{-1}$	2.6	2.5	2.5	2.4	2.5	2.4	2.3	2.4	2.1-2.6
phosphate $mmol.l^{-1}$	0.9	0.7	0.8	1.2	0.8	1.2	1.0	0.9	0.8-1.4
haemoglobin $g.dl^{-1}$	17.3	15.8	15.4	15.9	14.3	15.8	15.5	15.4	13-18
white blood cells x $10^{9}.l^{-1}$	5.8	10.1	4.4	6.4	6.2	5.6	5.3	5.0	4-11
platelets x $10^{9}.l^{-1}$	226	281	285	245	176	217	230	238	150-350

blood samples were taken at 75, 105 and 150 min and fluid was replaced according to the urinary volumes as described above once the infusion was complete.

Samples

All samples were processed as described in Chapter 2.

Drug assay

Plasma and urinary concentrations of frusemide were measured by HPLC as described in Chapter 2.

Pharmacokinetic analysis

The plasma concentration-time data were analysed using the SIPHAR pharmacokinetic programme as described in Chapter 2.

Due to the low plasma concentrations following oral frusemide, accurate curve fitting was not possible and the analysis was therefore restricted. The elimination half life, $t_{½}$, was estimated by visual inspection of the plasma concentration time curve with peeling of the terminal phase. The $AUC_{0-\infty}$ was calculated by the trapezoidal rule and the bioavailability as the ratio of the $AUC_{0-\infty}$ after the oral and intravenous administration of frusemide.

The plasma concentration-time curves following the intravenous dose were best described by a two compartment model comprising a distribution and elimination phase. Initial parameters were estimated by peeling and refined by iterative analysis. The goodness of the fit was assessed using the coefficient of variation of each parameter and the values are presented in Table 6.2. In addition, model independent analysis was used to estimate $t_{½}$, $AUC_{0-\infty}$, total clearance and V_d following the intravenous infusion.

Table 6.2. The coefficients of variation (CV%) for the parameters obtained by analysis of the plasma concentration-time data following the infusion of 40 mg frusemide intravenously over one hour to 8 healthy male volunteers. The data were best described by a two compartment model. Initial parameters were estimated by peeling and refined by iterative analysis. The distribution process is represented by intercept "D" and slope "d" and the elimination phase by intercept "B" and slope "ß" as described in Chapter 2.

	Distribution phase		Elimination phase	
No.	Intercept "D" (CV%)	Slope "d" (CV%)	Intercept "B" (CV%)	Slope "ß" (CV%)
1	21.0	49.1	10.3	5.8
2	25.7	46.4	45.0	23.4
3	10.5	23.5	13.0	8.6
4	6.2	14.2	20.9	13.2
5	15.9	24.6	7.6	5.1
6	8.8	15.2	47.5	26.6
7	3.7	5.5	28.2	18.3
8	8.6	14.8	47.8	24.9

A modified version of the Wagner Nelson method was used to estimate the fraction of the oral dose absorbed at each time point as described in Chapter 2. Thus

$$F_t = \frac{AUC_{0-t} + C_t/K}{AUC_{0-\infty}}$$

where AUC_{0-t}, C_t were from the data obtained following the oral dose of frusemide and K and $AUC_{0-\infty}$ from the intravenous data. The latter represents the maximum AUC that would have been achieved if all of the oral dose had been absorbed.

Total clearance was calculated as intravenous dose divided by $AUC_{0-\infty}$ and the renal clearance as the amount of frusemide (oral or intravenous) excreted 24 h divided by the corresponding $AUC_{0-\infty}$ as described in Chapter 2. The non-renal clearance was estimated as the difference between the total and the renal clearance following the intravenous dose. Bioavailability was also calculated from the ratio of the urinary recoveries following the oral and intravenous dose respectively.

Statistics

Correlation and linear regression were used to establish the relationship between parameters estimated following the oral and intravenous administration of frusemide and the significance of any observed differences was determined by the Wilcoxon test.

SECTION 6.3: RESULTS

Plasma concentrations of frusemide

The individual plasma concentration-time curves following the oral administration and intravenous infusion of 40 mg frusemide in the 8 volunteers are shown in Fig. 6.1 and Fig. 6.2 respectively and the mean concentrations are shown in Fig. 6.3. Subject number 6 had a higher AUC after oral than intravenous frusemide and this may reflect inaccuracies due to an interfering peak which was visible on the blank sample. The data were therefore not included in the analysis.

The absorption and distribution of frusemide

The oral absorption of frusemide was characterised by marked inter-subject variability (Fig. 6.1) and there were striking differences in individual values for C_{max}, T_{max} and AUC (Table 6.3). The mean C_{max} was 1.49 $\pm$0.82 mg.l^{-1} and the T_{max} ranged from 60 to 120 min with a mean value of 90 min. The $AUC_{0-\infty}$ was also variable with a mean value of 171 $\pm$86 min.mg.l^{-1} and the bioavailability of oral frusemide calculated from the AUC ranged from 27.6 to 84.3% with a mean of 53.6% (Table 6.3).

The mean fraction of the dose absorbed at each time point is shown on Figure 6.4. Following the oral administration of frusemide just under 30% of the dose had been absorbed at 60 min (26.7 $\pm$28.8). By 90 min the absorption process was complete with 53.3 $\pm$24.2% of the dose absorbed corresponding to the mean bioavailability.

As expected, the individual plasma concentration-time curves were more uniform following the intravenous infusion of frusemide (Fig. 6.2). In this case, the mean C_{max} was 3.34 $\pm$0.54 mg.l^{-1} and the mean $AUC_{0-\infty}$ was 301 $\pm$58 min.mg.l^{-1} (Table 6.4). Frusemide had a small volume of distribution (V_d) of 14.1 $\pm$3.8 l.

Table 6.3. Maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the plasma concentration time curve ($AUC_{0-\infty}$), bioavailability and half life of elimination ($t_{½}$) of frusemide in 7 healthy male volunteers following the oral administration of 40 mg. Bioavailability was calculated as the ratio of $AUC_{0-\infty}$ after oral and intravenous administration of the same dose on different occasions.

Subject	C_{max} (mg/l)	T_{max} (min)	$AUC_{0-\infty}$ (min.mg/l)	Bioavailability (%)	$t_{½}$ (min)
1	2.79	90	328	84.3	63.5
2	2.37	90	192	62.9	45.7
3	1.97	60	242	77.9	69.5
4	1.01	90	155	57.0	82.4
5	0.98	120	136	35.4	77.9
7	0.79	90	78	27.6	53.0
8	0.49	90	63	30.4	63.8
mean	1.49	90	171	53.6	65.1
±sd	0.82	16	86	21.3	12.0

Table 6.4. Maximum plasma concentration (C_{max}), area under the plasma concentration time curve ($AUC_{0-\infty}$), Volume of distribution (V_d), plasma half life of elimination ($t_{½}$) and total clearance of frusemide in 8 healthy male volunteers given 40 mg by intravenous infusion over one hour.

No.	C_{max} (mg/l)	AUC (min.mg/l)	V_d (l)	$t_{½}el$ (min)	Total Clearance (ml/min)
1	4.12	389	9.0	60.5	102.9
2	3.75	306	9.5	50.4	130.9
3	3.06	311	12.4	66.7	128.6
4	2.88	271	14.2	66.8	147.5
5	4.13	385	8.7	58.1	103.7
6	2.88	254	20.9	92.0	157.1
7	3.23	283	16.9	82.8	141.4
8	2.70	210	14.5	52.9	190.6
mean	3.34	301	14.1	66.3	137.8
±sd	0.54	57	3.8	13.5	26.9

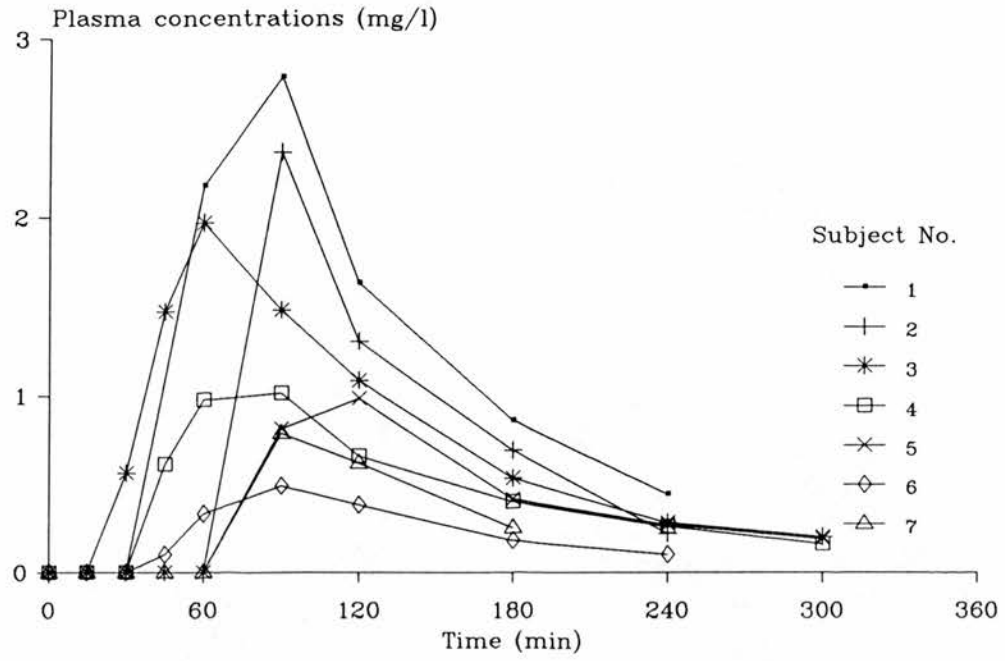


Fig 6.1. Plasma concentrations of frusemide in 7 healthy volunteers following the administration of 40 mg orally.

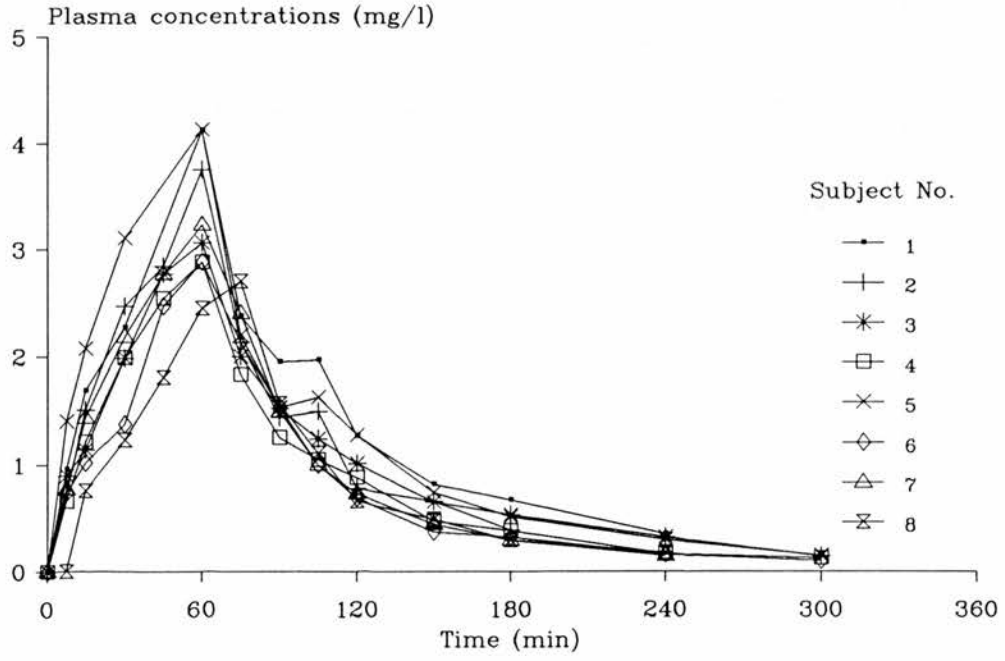


Fig 6.2. Plasma concentrations of frusemide in 8 healthy volunteers following the administration of 40 mg by intravenous infusion over 1 h.

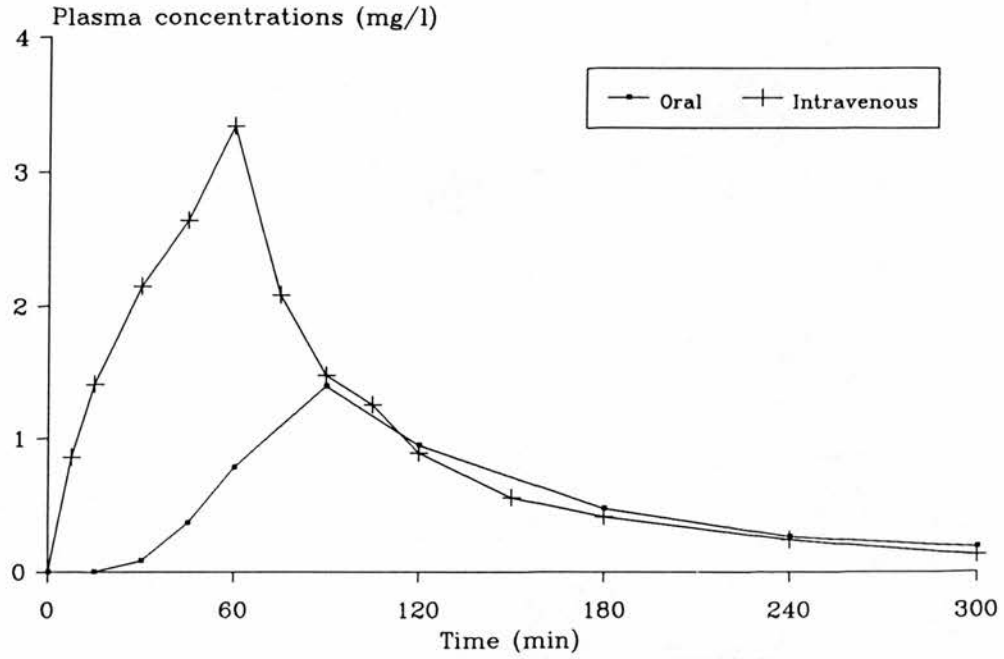


Fig 6.3. The mean plasma concentrations of frusemide in 8 healthy volunteers following the oral administration (n=7) and intravenous infusion over 1 h of 40 mg.

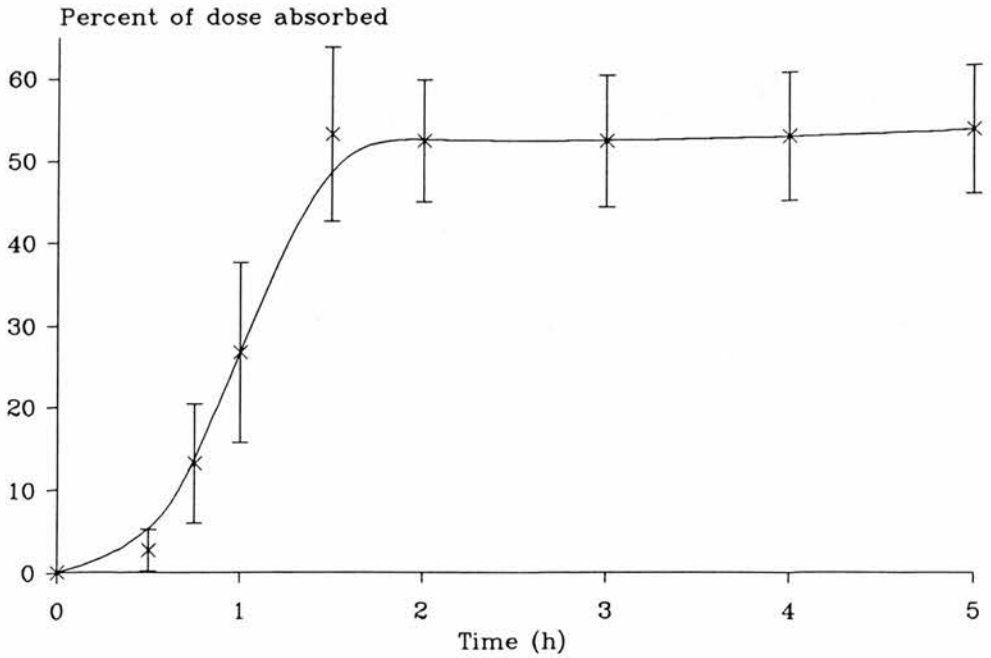


Fig 6.4. The fractional absorption 40 mg oral frusemide (mean ±s.e.m.) in 7 healthy volunteers.

Elimination of frusemide

The mean $t_{½}$ calculated by peeling was 65.1 ±12.0 min after oral frusemide and 60.3 ±13.4 min after the intravenous infusion. The values were positively correlated ($r = 0.77$, $p<0.05$, $n=7$) and 3 of the 7 volunteers had lower $t_{½}$ values following intravenous frusemide. Using the fitted model, the $t_{½}$ of frusemide was 66.3 ±13.5 min after the intravenous infusion (Table 6.4). The total clearance of frusemide was 137.8 ±26.9 $ml.min^{-1}$.

The renal excretion of frusemide

When frusemide was administered orally the mean recovery in the urine was 13.9 ±3.6 mg or 34.7 ±8.9% of the dose (Table 6.5). Following the intravenous dose the recovery was higher at 22.1 ±1.9 mg or 55.2 ±4.8% of the total dose infused (Fig. 6.5).

As expected, the time course of delivery of frusemide into the urine differed with both routes as shown in Fig. 6.6. Little frusemide was excreted in the first hour after oral dosing (<3%). Most of the amount excreted was recovered between 1 to 2 h (14.9 ±7.3%) and from 2 to 4 h (11.33 ±2.4%) after which the excretion rate declined sharply (Fig. 6.6). By contrast, over 25% of the total dose was excreted during the hour-long intravenous infusion of frusemide (26.1 ±3.3%) and in the subsequent two hours a further 20% was eliminated (21.1 ±9.1%). The amount excreted then fell sharply and was less at each collection point than following the oral dose (Fig. 6.6). These differences presumably reflect the relatively slow and incomplete absorption of oral frusemide.

Table 6.5. Twenty four hour urinary recovery, renal clearance and bioavailability of frusemide in 8 healthy male volunteers given 40 mg frusemide orally and intravenously and the calculated non renal clearance of the intravenous dose. The bioavailability is calculated as the ratio of the twenty four hour urinary recovery following the oral and intravenous doses.

Subject	Frusemide recovery (mg)	Renal clearance (ml/min)	Non-renal clearance (ml/min)	Bioavailability (%)
1	21.1	64.4		95.6
1*	22.1	56.8	46.2	
2	15.4	80.0		69.2
2*	22.2	72.7	58.2	
3	14.2	58.6		58.8
3*	24.2	77.7	50.9	
4	11.0	71.4		56.6
4*	19.5	71.9	75.6	
5	12.6	92.1		65.7
5*	19.1	49.6	54.1	
6	16.6			66.1
6*	25.2	98.9	58.3	
7	10.2	131.3		47.0
7*	21.8	76.9	64.4	
8	9.9	154.5		43.8
8*	22.5	107.1	83.5	
mean	13.9	93.2**		62.9
std	3.6	33.6		15.0
mean*	22.1	76.5	61.4	
std*	1.9	18.0	11.8	

* = intravenous
** = (n=7)

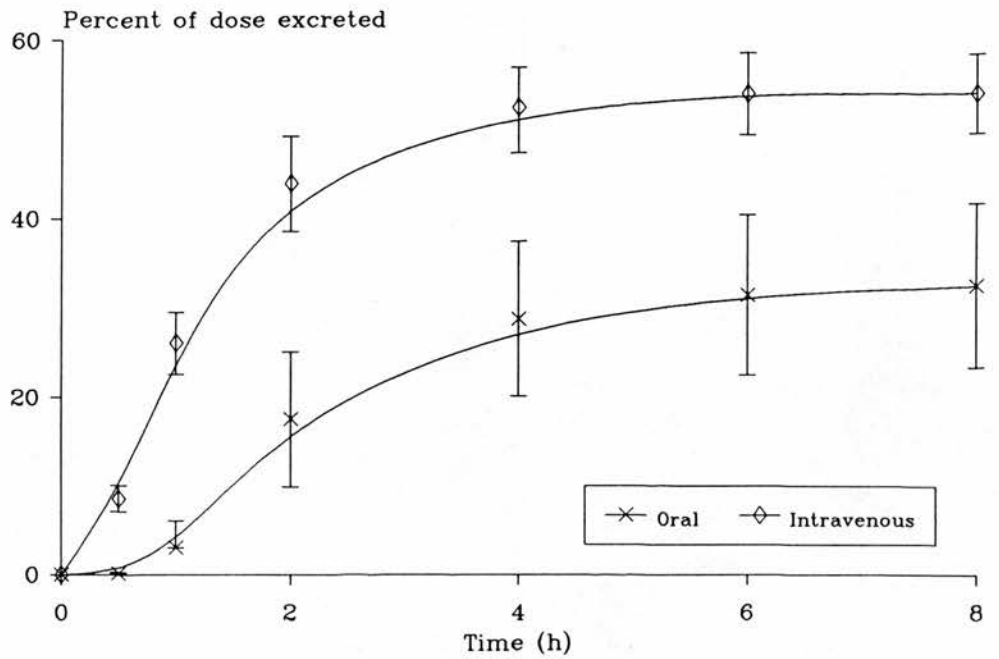


Fig 6.5. The cumulative urinary excretion of frusemide following 40 mg orally and by intravenous infusion over 1 h in 8 healthy volunteers.

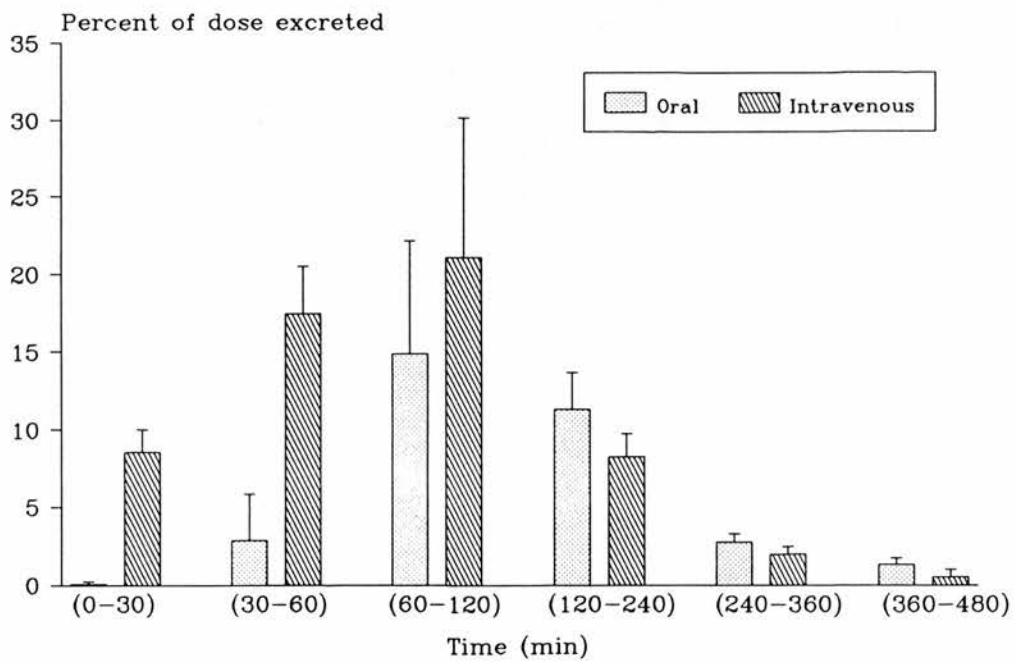


Fig 6.6. The rate of excretion of frusemide in 8 healthy volunteers following oral and intravenous administration of 40 mg.

The total urinary recovery following the oral dose was positively correlated with the bioavailability calculated from $AUC_{0-\infty}$ values ($r = 0.82$, $p<0.05$, $n=7$). The mean bioavailability of frusemide calculated from the ratio of its urinary recoveries following oral and intravenous administration was 62.9 $\pm$15.0% and this was slighty higher than the corresponding value estimated from the $AUC_{0-\infty}$ (53.6 $\pm$21.3%, $p=0.2$).

The renal clearance of frusemide was 93.2 $\pm$33.6 $ml.min^{-1}$ following the oral dose and 76.5 $\pm$18.0 $ml.min^{-1}$ following the intravenous infusion but the differences were not significant.

The non-renal clearance of frusemide

The non-renal clearance after intravenous frusemide was 61.4 $\pm$11.8 $ml.min^{-1}$, or just less than half of the total clearance (Table 6.5). In two of the 8 subjects the non-renal clearance exceeded the renal clearance as shown in Figure 6.7.

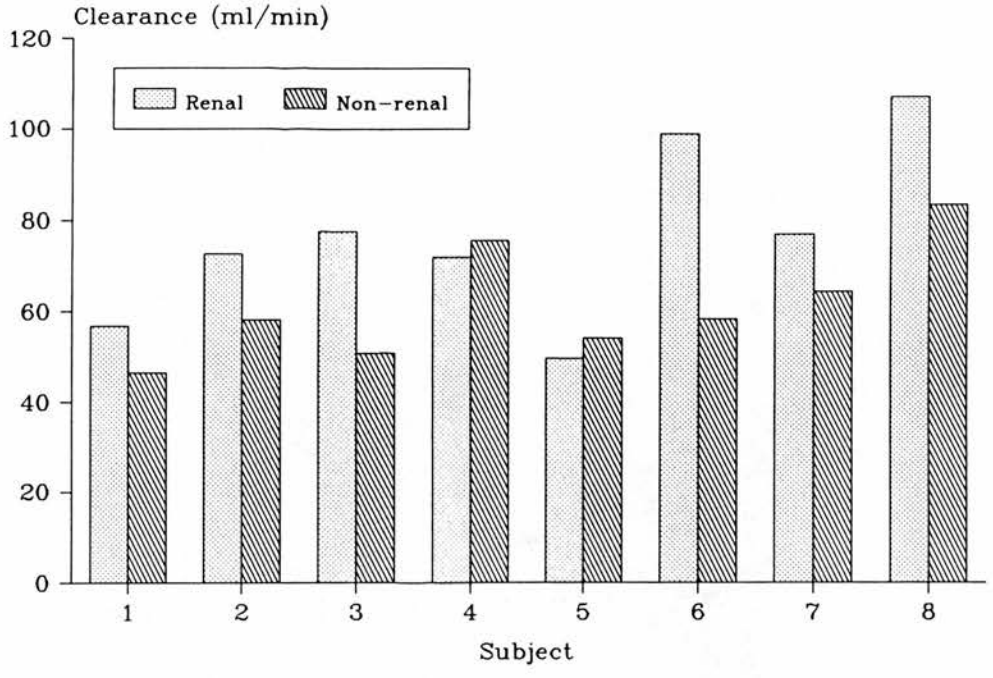


Fig 6.7. The individual renal and non-renal clearances of frusemide following the intravenous infusion of 40 mg in 8 healthy volunteers.

SECTION 6.4: DISCUSSION

When frusemide was administered orally to healthy fasting male volunteers the pattern of absorption differed between individuals and the C_{max}, T_{max} and bioavailability were very variable. The absorption of frusemide is known to be erratic (Boles Ponto and Schoenwald, 1990) and reported values of the T_{max} and bioavailability of oral frusemide in healthy volunteers are summarised in Table 6.6. In general the T_{max} is reported to be between 60 and 90 min (Beermann et al., 1975, Branch et al., 1977, Kelly et al., 1974 and Waller et al., 1982, Table 6.6) although values may be much higher or lower (Hammarlund et al., 1984). The bioavailability of frusemide calculated from urinary recoveries or AUC values has been reported in a number of studies and is also characterised by a high degree of variability both within and between studies, ranging from 20 to 100 % in young volunteers (Table 6.6). In the present study the T_{max} varied from 60 to 120 min and the bioavailability from 28 to 84% when calculated from the AUC.

The reasons proposed to explain the erratic and variable absorption of frusemide include inter- and intra-subject variability, the use of different dosage forms and different study protocols including whether the subjects were in a fasting or a non-fasting state (Boles Ponto and Schoenwald, 1990). The methods used to measure frusemide are also potential sources of variability (Benet, 1979).

The rate and extent of absorption of oral frusemide may vary when different formulations are compared such as solutions, tablets or immediate and sustained release preparations. In healthy subjects the rate of absorption of frusemide in solution was faster than the equivalent dose given as a tablet but there was no difference in overall bioavailability (Hammurlund et al., 1984). Similarly, Kelly et al., 1974, found no differences in the absorption of a solution or a tablet in fasting volunteers. The absorp-

tion of tablets made by different manufacturers has also been compared. In one study, seven marketed brands of frusemide 40 mg were compared and only one brand was considered to be inequivalent to the other six on the basis of greater inter-subject variability in the plasma concentration-time curves and AUC values (Straughn et al., 1986). Similarly, there were no differences in the AUC or urinary recoveries of "Lasix" and "Impugan", two marketed brands of frusemide (Beermann et al., 1978). However comparison of a branded and generic formulation of frusemide suggested that the latter was significantly less bioavailable (Martin et al., 1984) and sustained release preparations may have reduced bioavailability when compared to conventional tablets (Beermann, 1982). It is therefore unclear to what extent the reported erratic and variable absorption of frusemide can be explained by the use of different formulations. In the present study all volunteers were given a standard 40 mg tablet of frusemide manufactured by Hoechst ("Lasix").

The fasting or non-fasting state is another factor which may lead to variability . Postprandial administration delayed the appearance of frusemide in the plasma with a lower C_{max} and more prolonged concentrations but no differences in overall bioavailability (Kelly et al., 1974). In contrast, Beermann and Midskow (1986) found a reduced but parallel plasma concentration time profile in the fasting and non-fasting states with a 30 % reduction in bioavailability. Hammarlund et al., (1984), found that while the presence of food in the stomach dramatically changed the shapes of the plasma concentration-time curves for most individuals with a reduction in C_{max} and a delay in T_{max}, it did appear to diminish the interindividual variability. Food intake slighty reduced the bioavailability but not to a significant extent. In the present study all subjects fasted for the first hour after frusemide administration.

Secondary or multiple peaks may occur in the plasma following the administration of frusemide and this has been explained on the basis of enterohepatic cycling (Waller et

al., 1982 and Smith et al., 1980 (a)). However, the impact of this small amount of drug has been discounted since on average 50% of the dose remains available for absorption in the gastrointestinal tract as judged by the bioavaliability (Hammurlund et al., 1984). Furthermore, in the rat 20 to 30% of an oral dose of frusemide is metabolised in the gut wall and stomach during absorption and hepatic elimination accounts for less than 10% (Lee and Chiou, 1983). The findings suggested that metabolism of frusemide in the gastrointestinal tract may account for at least some of the erratic absorption that occurs with oral dosing.

Frusemide may also exhibit site-specific absorption. As a weak acid it could be absorbed from the stomach as well as from the intestine where most weak acids are absorbed because of the larger absorptive area (Hammurlund et al., 1984). In the rat the stomach has a greater absorptive capacity for frusemide than the intestine despite its smaller surface area (Chungi et al., 1979).

The role of the stomach as an absorptive site for frusemide is complicated however by the fact that it is also a potential site for frusemide metabolism (Lee and Chiou, 1983). Furthermore, although food will delay gastric emptying allowing the drug to remain in the stomach longer, it will also buffer the pH and slow absorption (Boles Ponto and Schoenwald, 1990). At the moment there is no conclusive evidence to suggest that frusemide is primarily absorbed in the stomach in humans but inter and intra individual variation in gastric emptying could explain some of the variability in frusemide absorption from the small intestine.

Finally, the variable absorption patterns found in different studies may be partly due to problems with the different assays used to measure frusemide (Benet, 1979). The most consistent results are obtained with HPLC (Benet, 1979 and Boles Ponto and Schoenwald, 1990).

Following intravenous frusemide the plasma concentrations were best described by a two compartment model. This model is probably the most appropriate from a pharmacokinetic perspective (Cutler and Blair, 1979). The high plasma protein binding and low lipid solubility of frusemide restricts its distribution and the reported values of V_d are 2 to 5 times the plasma volume which is 2.8 to 3.5 l in a 70 kg man (Diem and Lentner, 1970). The V_d of just over 14 l in the present study is therefore consistent with the previous reports listed in Table 6.7.

The reported values for the plasma half life of frusemide in healthy subjects are also variable but usually fall in the range of 30 to 120 min (Table 6.7). The progressively longer values reported in more recent studies may be due to longer sampling times made possible by more sensitive assays (Boles Ponto and Schoenwald, 1990). A three compartment model has been used for analysis with estimation of the $t_{½}$ from the terminal phase. However, this segment represents only 10 % of the AUC and plasma concentrations at this time are well below those which would produce pharmacologically active urinary concentrations of frusemide (Hammarlund et al., 1984). The problem may be compounded by the use of different methods of curve fitting (Chennavasin et al., 1981). Using equal weighting factors gives shorter values for $t_{½}$ than using weighting factors giving more weight to lower concentrations of the drug because with the former the terminal portion of the curve is ignored.

The reported total plasma clearance of frusemide in healthy volunteers has ranged from 80 to 288 $ml.min^{-1}$ with an average value of about 170 $ml.min^{-1}$ (Table 6.6) Renal and non-renal processes contribute in highly variable but often equivalent magnitude to the total clearance (Table 6.7). This is in agreement with the results of the present study where the renal clearance contributed just over half the total clearance of almost 140 $ml.min^{-1}$.

The reasons for the widely different values for total clearance quoted in the literature are unlikely to be related to assay sensitivity or methods of curve fitting (Chennevasin et al., 1981). The clearance of frusemide is calculated from the $AUC_{0-\infty}$ and the terminal portion of the tail (which is the only estimated part), normally contributes little to the overall AUC. The plasma clearance of frusemide was similar in smokers and non smokers although the latter tended to have a higher non renal clearance (Lambert et al., 1983). The renal clearance is thought not to be affected by dose (Waller et al., 1985) or route of administration (Smith et al., 1980 (a)) but is reduced in the elderly presumably because of the decline in renal function in such individuals (Andreason et al., 1983, Kerremans et al., 1983).

Since the diuretic site of action of frusemide is primarily at the luminal surface of the ascending limb of the loop of Henle, the fraction of the dose excreted unchanged in the urine represents the amount available for pharmacological action (Brater, 1986). This appears to be variable and after an intravenous dose of frusemide approximately 50 to 80 % of the dose will be excreted in the urine but after oral administration the corresponding fraction is only 20 to 55 % (Tables 6.6 & 6.7). In the present study the mean amounts were 55 and 34 % respectively. The amount of frusemide recovered in the urine can also be used to measure bioavailability (Waller et al., 1982) and in the present study this gave a value of 63%.

The present study confirms the previously reported variable absorption of oral frusemide in healthy fasting volunteers. Frusemide had a small volume of distribution presumably due to its polarity and high plasma protein binding and its disposition after intravenous infusion was best described by a two compartment model. About half of the intravenous dose and one third of the oral dose was available for pharmacological action as judged by the fraction excreted in the urine. The non-renal clearance accounted for about half of the overall plasma clearance.

Table 6.6. Pharmacokinetic variables of oral frusemide in healthy fasting volunteers.

Author	Year	Dose (mg)	Assay	No.	T_{max} (mins)	Bioavailability (%)	f_{ex} (%)
Beerman	1975	7-40	^{35}S	7	45-75		49
Beerman	1978	40	GLC + HPLC	5	48 ±19*		41 ±11*
					105 ±73		46 ±15
Beerman	1982	40	HPLC	12	90		37 ±9
Beerman	1986	40	GLC	10	84		29 ±6
Branch	1977	80	FL +TLC	6	90	50	31 ±4
Brater	1982	40	HPLC	25		38 ±20	23 ±15
Brater	1983	20, 40, 80	HPLC	10			30
Hammarlund	1894	40	HPLC	8	30-143	51 ±7	40 ±2
Kelly	1974	80	FL	8	60-70	60	30-50
Kelly	1977	240	^{35}S	2		30, 42	
Rane	1978	80	HPLC	6		63 ±9	
Smith	1980 (a)	80	HPLC	9		43 ±10	28 ±8
Tilestone	1978	44	^{35}S	5		69 ±7	
Waller	1982	40	HPLC	18	50 ±24**	64 ±22**	41 ±2**
					86 ±50	71 ±35	44 ±15
Zhu	1987	10-40	HPLC	6		83 ±14	60-75

* comparison of 2 different brands of frusemide, ** comparison of solution and tablet of frusemide, f_{ex} = fraction of the dose excreted, GLC = gas liquid chromatography, Fl = fluorometric assay, ^{35}S = radiolabelled frusemide

Table 6.7. Pharmacokinetic variables in healthy volunteers given single doses of intravenous frusemide.

Author	Year	Dose (mg)	Assay	No.	$t_{\frac{1}{2}}$ (mins)	Cl_p ($ml.min^{-1}$)	Cl_r ($ml.min^{-1}$)	Cl_{nr} ($ml.min^{-1}$)	f_{ex} (%)	V_d (l)
Alván	1988	0.5*	HPLC	6	48 ±7	177 ±41	116 ±30	61 ±25	66 ±9	240 ±60**
Andreasen	1977	40	FL + TLC	8	72	166 ±42	116 ±79	50	63 ±25	17.2 ±4.64
Andreasen	1978	40	Fl + TLC	7		219 ±49				7.2 ±2.0
Andreasen	1982	80	HPLC	10	70 ±20	170 ±19	114 ±33	56 ±18	45 ±20	
Beerman	1975	7	^{35}S	2	47, 53				82, 84	
Beerman	1977	40	GLC + HPLC	5	52 ±15	194 ±35	95 ±24	99 ±48		210 ±56*
Branch	1977	80	FL	6	50	125	75	50	65	11.9
Cutler	1974	.5-1.5*	Fl	4	30 ±6	162 ±38	149 ±37	12 ±1	92	115 ±23**
Fuller	1981	10	HPLC	3	42	185			49	119**
González	1982	40	FL	7	44 ±13	167 ±54				10.4 ±1.5
Hammarlund	1984	40	HPLC	8	36	162 ±11	117 ±11	45	71 ±2.7	8.5
Homeida	1977	40	Fl	6	38 ±3	268 ±19	90 ±10	178 ±2		14.9 ±1.3
Honari	1977	1*	HPLC	4	36 ±5	155 ±24	134 ±23	21 ±3	87 ±3	12.3 ±1.8
Keller	1981	40	GLC	7	51 ±8	174 ±32	118 ±30	56 ±28	70	12.7 ±2.4
Kelly	1974	80	FL	4	26 ±10	142 ±39				5.0 ±0.9
Kelly	1977	240	^{35}S	2	43,30	194,109	150,71	44,38		16.4 ±9.3
Lambert	1983	40		5	172 ±31	96 ±8	72 ±7	12 ±2		12.0 ±2.3
Rane	1978	80	HPLC	6	51 ±4	158 ±11	80	77	51	110 ±7**
Smith (a)	1980	40	HPLC	9	92 ±7	164 ±26	110 ±24	54 ±10	66 ±7	109 ±19**
Smith (b)	1980	40	HPLC	4	82 ±5	160 ±15	118 ±17	42 ±12	74 ±7	8.4 ±0.9
Tilstone	1978	22	^{35}S	11	48 ±4	112				
Verbeek	1982	80	HPLC	10	60 ±6	156 ±7	87 ±8	69 ±8	59 ±3	8.5 ±0.4
Waller	1982	40	HPLC	18	78	117 ±41	88 ±45	32 ±12		130 ±60**
Zhu	1987	20	HPLC	6	77 ±12	127 ±38	107 ±30	19	89 ±4	8.5 ±2.2

*$mg.kg^{-1}$, **$ml.kg^{-1}$, $Cl_{p,r,nr}$=plasma, renal and non-renal clearance, f_{ex}=fraction of dose excreted, FL=fluorometric assay, TLC=thin layer chromatography, GLC=gas liquid chromatography, ^{35}S=radiolabelled

CHAPTER 7

STUDIES WITH SINGLE DOSES OF FRUSEMIDE IN PATIENTS WITH END-STAGE RENAL FAILURE MAINTAINED ON CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD)

SECTION 7.1: INTRODUCTION

Frusemide is a powerful diuretic and one of its major advantages is its ability to induce diuresis even in patients with advanced renal impairment (Allison and Kennedy, 1971). It is therefore extensively used in the treatment of patients with chronic renal failure but large doses are often required to cause a diuresis (Brater et al., 1986). Studies have shown that frusemide must gain access to the tubular lumen of the kidney to cause an effect (Chennavasin et al., 1979). Frusemide is highly bound to plasma proteins so this access occurs by active secretion into the lumen by the organic acid secretory pump of the proximal tubule (Odlind, 1983). In patients with chronic renal failure endogenous organic acids accumulate and block frusemide secretion (Rose et al., 1976). Larger doses are therefore required to force sufficient amounts of drug into the lumen to cause an effect.

The disposition of frusemide has been extensively studied in patients with chronic renal failure following both oral and intravenous administration (Tables 7.10 and 7.11) but the results are often conflicting (Benet, 1979). This may partly reflect differences in study protocols including the assays used, the nature and degree of renal impairment, the dose of frusemide or inter-individual differences in the handling of frusemide as discussed for the normal volunteers.

The object of this study was to determine the disposition of frusemide in a group of patients with end-stage renal failure maintained on CAPD who were not taking frusemide as part of their regular medication. Little specific information is available on drug absorption and bioavailability in peritoneal dialysis but it is generally accepted that the gastrointestinal disturbances associated with end-stage renal disease may in some way affect drug absorption in these patients (Paton et al., 1985). The renal clearance of all drugs including frusemide should be minimal and clearance by non-

renal mechanisms may include elimination across the peritoneal membrane. It was thus hoped to characterise the disposition of frusemide in patients with end-stage renal failure and to determine the extent of non-renal clearance including peritoneal clearance in these patients.

SECTION 7.2: METHODS

Patients

Eleven patients (3 female, 8 male) with end-stage renal failure (creatinine clearance < 5 ml.min^{-1}) maintained on continuous ambulatory peritoneal dialysis (CAPD) were studied on 2 separate occasions. Their mean age was 66 yr (range 57 - 73) and weight 64 kg (range 48 - 74). Details of the medical histories and medication are presented in Table 7.1 and results of haematology and biochemistry in Tables 7.2 and 7.3. None of the patients were being treated with frusemide at the time of the study and patients with a history of peritonitis in the previous 3 months were excluded as were patients with haemoglobin concentration of less than 7 g.dl^{-1}.

All patients performed four peritoneal exchanges each day with 2000 ml of standard dialysate solutions via indwelling Tenckhoff catheters as described in Chapter 2. Details of the patients' dialysis regimes and daily fluid allowances are given in Table 7.4.

Experimental Design

The patients were studied on 2 separate occasions at least a week apart. On the morning of the first study day the patients came to the CAPD training room at 08.30 h with their overnight dialysate still in the peritoneal cavity. The weight and glucose content of a new bag of dialysate was noted and the patients then performed the first exchange of the day. An intravenous cannula was inserted in a forearm vein and 80

mg frusemide (2 x 40 mg tablets, Hoechst, U.K. Limited) was given orally with 100 ml of water. The patients then remained recumbent for an hour.

Following this, the patients were allowed their normal fluid intake (Table 7.4) throughout the day. Breakfast was served one hour after the dose of frusemide when the patients took their normal medications (Table 7.1) and lunch was taken at 13.00 h according to their usual dietary requirements.

Venous blood (3ml) was sampled before the administration of the frusemide and at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after. The used dialysate bags of that day were kept and the volumes measured. All urine passed during the 24 h period was collected. The patients were free to go home after their 3rd exchange of the day at approximately 17.30 h.

A week later the study was completed. On this occasion frusemide 80 mg was infused intravenously over one hour at a continuous rate in 50 or 100 ml of 0.9% saline (depending on the daily fluid allowance) via an "IMED 960" volumetric infusion pump. Blood sampling was the same as described above with extra samples taken at 75, 105 and 150 min after the start of the infusion.

Samples

All samples were processed as described in Chapter 2.

Drug assay

Plasma, urine and dialysate concentrations of frusemide were measured by HPLC as described in Chapter 2.

Table 7.1: Clinical details of 11 patients with end stage renal failure maintained on CAPD.

Patient No.	Age sex	Medical diagnosis	Regular drug therapy
1	58 F	pyelonephritis asthma angina pectoris	prednisolone 5 mg daily ranitidine 75 mg BD "gaviscon" aluminium hydroxide 475 mg TID
2	70 F	glomerulonephritis	ferrous sulphate 320 mg daily folic acid 5 mg twice per week
3	67 M	pyelonephritis nephrocalcinosis hypertension	metoprolol 25 mg BD aluminium hydroxide 475 mg TID "Fefol vit" 1 TID
4	73 M	glomerulonephritis coeliac disease hypothroidism	alfacalcidol 0.25 μg daily "Fefol vit" 2 daily aluminium hydroxide 950 mg TID thyroxine 100 μg daily
5	68 M	Goodpasture's Syndrome ischaemic heart disease	"Fefol vit" 2 daily isosorbide mononitrate 20 mg BD aluminium hydroxide 475 mg TID
6	67 F	polycystic kidneys hypertension angina pectoris pernicious anaemia	alfacalcidol 0.25 μg daily "Fefol vit" 2 daily metoprolol 50 mg BD ranitidine 150 mg nocte
7	57 M	polyctstic kidneys hypertension	"Fefol vit" 2 daily nifedipine slow release 20 mg BD metoprolol 50 mg BD
8	67 M	hypertensive neph ropathy	aluminium hydroxide 950 mg BD "Fefol vit" 2 daily aspirin 375 mg daily
9	67 M	glomerulonephritis hypertension transient ischaemic attacks	aluminium hydroxide 950 mg TID nifedipine slow release 20 mg BD "Fefol vit" 2 daily metoprolol 25 mg BD aspirin 375 mg daily ranitidine 75 mg nocte
10	64 M	Diabetes mellitus type 2 chronic renal failure ?cause hypertension	aluminium hydroxide 475 mg daily "Fefol vit" 2 daily metoprolol 25 mg BD digoxin 0.0625 mg daily gliclazide 40 mg BD
11	70 M	glomerulonephritis ischaemic heart disease hypertension	diltiazem 60 mg TID "Fefol vit" 2 daily captopril 25 mg BD prasozin 2 mg TID alfacalcidol 0.5 μg daily aluminium hydroxide 475 mg TID

Table 7.2. Clinical biochemical test results in 11 patients with end stage renal failure maintained on continuous amulatory peritoneal dialysis.

Patient No.	Protein $g.l^{-1}$	Albumin $g.l^{-1}$	Calcium $mmol.l^{-1}$	Phosphate $mmol.l^{-1}$	Alkaline phosphatase $u.l^{-1}$	Bilirubin $\mu mol.l^{-1}$	ALT $u.l^{-1}$	GGT $u.l^{-1}$	Bicarbonate $mmol.l^{-1}$	Creatinine $\mu mol.l^{-1}$	Urea $mmol.l^{-1}$
1	69	42	2.7	1.4	176	3	17	19	21	1066	28.3
2	59	34	2.5	1.5	156	4	14	13	25	1094	14.9
3	58	33	2.6	1.4	125	6	29	21	27	956	16.6
4	71	38	2.2	2.5	77	5	20	39	21	1276	20.0
5	50	31	2.0	1.6	60	3	11	10	25	1022	21.0
6	68	34	2.4	1.8	298	6	26	53	24	859	16.5
7	70	42	2.3	1.4	56	9	31	34	20	844	13.5
8	59	28	2.1	1.8	114	3	19	8	21	939	17.4
9	74	37	2.4	1.7	101	4	25	23	25	1139	26.2
10	66	39	2.3	1.7	124	10	35	53	20	1326	28.9
11	59	33	2.1	1.3	115	4	18	23	20	1060	19.9
Normal range	60-80	36-47	2.1-2.6	0.8-1.4	40-100	2-17	10-40	10-55	24-30	55-150	2.5-6.6

Table 7.3. Results of haematological tests in 11 patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis.

Patient No.	Haemoglobin concentration $g.l^{-1}$	white cell count $x\ 10^9.l^{-1}$	platelets $x\ 10^9.l^{-1}$
1	12.0	8.3	218
2	7.2	5.4	302
3	8.8	5.1	190
4	9.7	8.6	314
5	8.1	10.5	296
6	10.6	4.2	260
7	17.9	6.3	180
8	7.5	6.0	328
9	10.9	6.9	201
10	10.7	5.4	209
11	9.2	4.1	199
Normal range	13-18	4-11	150-350

Table 7:4. Details of the daily fluid allowance and dialysis regimes of 11 patients with chronic renal failure maintained on continuous ambulatory peritoneal dialysis.

Patient No.	Daily fluid intake (ml)	Time on CAPD	Exchanges per day	Glucose content of each bag (%)
1	500	4 years	4	2.27 x 4
2	1500	10 years	4	2.3 x 4
3	750	7 years	4	1.5 x 4
4	1000	7 months	4	2.27 x 2 3.86 x 2
5	750	6 months	4	2.27 x 1 1.36 x 3
6	500	2 years	4	2.27 x 3 1.36 x 1
7	750	7 months	4	2.27 x 2 1.36 x 2
8	750	2 years	4	2.27 x 4
9	1000	4 years	4	2.27 x 4 2.27 x 3
10	1500	3 years	4	3.86 x 1 2.27 x 3
11	1000	3 months	4	1.36 x 3 2.27 x 1

Pharmcokinetic analysis

The "Siphar" pharmacokinetic programme was used to analyse the plasma concentration-time data as described in Chapter 2.

The plasma concentration-time curves obtained with oral dosing were fitted to a one compartment model. Initial parameters were estimated by peeling and refined by iterative analysis. The goodness of the fit was established by the coefficients of variation of each parameter as described in Chapter 2 and these are listed in Table 7.5. These were less than 20-30% in all cases except for patient 4.

The plasma concentration-time data obtained following the intravenous dose was best described by either a one (patients 1, 8 and 9) or two (patients 2, 3, 5, 6, 10 and 11) compartment model. Coefficients of varaition of the estimated parameters were usually less than 20-30% (Table 7.6).

The fitted model was used to describe the disposition of oral and intravenous frusemide and to estimate the lag time and $t_{1/2}$. The Wagner-Nelson method was used to estimate the percent of the dose absorbed with time as described in Chapter 2. Model independent analysis was used to estimate the $AUC_{0-\infty}$, the total, renal and peritoneal clearances and the V_d.

Statistics

The significance of any observed differences was determined using the Students "t" test or the Wilcoxon or Mann-Whitney test for paired and un-paired data respectively, where approriate.

Table 7.5. The coefficients of variation (CV%) for the estimated parameters obtained by analysis of the plasma concentration time data following the administration of 80 mg frusemide orally to patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis. The data were best described by a one compartment model comprising an absorption and elimination phase. Initial parameters were estimated by peeling and refined by iterative analysis. The absorption phase is represented by intercept "A" and slope "α" and the elimination phase by intercept "B" and slope "ß" and described in Chapter 2.

	Absorption phase		Elimination phase	
No.	Intercept "A" (CV%)	Slope "α" (CV%)	Intercept "B" (CV%)	Slope "ß" (CV%)
1	0.9	30.6	13.7	20.1
2	3.4	31.5	14.3	13.8
3	10.2	12.6	7.5	5.6
4	3.7	41.2	19.5	21.3
5	4.9	24.1	10.8	17.3
6	0.9	14.7	2.7	10.4
7	3.1	24.6	9.4	15.4
8	3.7	26.7	10.3	19.8
9	0.6	11.6	0.8	6.1
10	0.8	29.1	11.3	21.0
11	0.2	13.1	0.2	8.6

Table 7.6. The coefficients of variation (CV%) for the estimated parameters obtained by analysis of the plasma concentration time data obtained following the administration of 80 mg frusemide intravenously to patients with end stage renal failure maintained on continuous ambulatory peritoneal dialysis. The data were best described by either a one or two (*) compartment model. The latter included a distribution and elimination phase. Initial parameters were estimated by peeling and refined by iterative analysis. The distribution phase is represented by intercept "D" and slope "d" and the elimination phase by intercept "B" and slope "ß" as described in Chapter 2. The data from patient 4 were not included in the analysis due to technical problems and patient 7 did not receive intravenous frusemide.

	Distribution phase		Elimination phase	
No.	Intercept "D" (CV%)	Slope "a" (CV%)	Intercept "B" (CV%)	Slope "ß" (CV%)
1			12.6	5.6
2*	29.1	37.9	3.8	6.2
3*	12.0	38.0	10.7	9.4
5*	7.4	14.1	21.2	28.8
6*	15.1	38.1	19.5	19.6
8			3.4	2.3
9			3.6	1.9
10*	17.8	30.3	6.0	10.0
11*	21.4	36.7	7.5	7.0

SECTION 7.3: RESULTS

Plasma concentrations of frusemide

The individual plasma concentration-time curves following the oral and intravenous administration of 80 mg frusemide are shown in Figures 7.1 and 7.2 respectively and the mean concentrations are shown in Figure 7.3. The intravenous data from patient 4 were thought to be inaccurate because of problems with venous access and patient 7 had a renal transplant before completing the second study day. The intravenous data are therefore presented for the other 9 patients.

The absorption and distribution of frusemide

The oral absorption of frusemide in patients on CAPD was characterised by marked intersubject variability (Figure 7.1). In most cases oral frusemide was absorbed slowly with a mean lag time derived from the raw data of almost 26 min (25.4 $\pm$26.4). However, two of the patients (patients 7 and 8) had much longer lag times and frusemide was not detected in the plasma until 90 and 120 min respectively (Table 7.7). In the other nine patients the lag time was zero in three, 15 min in three and 30 min in three. Using the fitted model the mean lag time was considerably shorter at 14.8 $\pm$9.1 min.

The mean C_{max} following the oral administration of frusemide to patients on CAPD was 2.91 $\pm$1.37 $mg.l^{-1}$ and the mean T_{max} was 126 min (range 60 to 240 min, Table 7.7). The $AUC_{0-\infty}$ was variable with a mean value of 1113 $\pm$440 $min.mg.l^{-1}$. The mean bioavailability was 70.1 $\pm$13.1% (n=9).

When the plasma concentration-time curves following oral frusemide were compared in healthy volunteers and patients with end-stage renal failure, it appeared that the absorption of frusemide in the latter was slower but more complete. Due to the low

plasma concentrations of frusemide measured in healthy volunteers following administration of 40 mg frusemide it was not possible to define the lag time with certainty. However, the T_{max} occurred earlier in the volunteers than the patients (90 $\pm$16 compared with 126 $\pm$58 min, respectively, NS). Despite this, the mean bioavailability of frusemide was higher in the patients with renal failure than in the volunteers although not significantly so (70.1 $\pm$13.1 and 53.6 $\pm$21.3 %, respectively).

A similar pattern was observed when the fraction of the dose absorbed with time was compared in the CAPD patients and the volunteers (Fig. 7.4). The patients had absorbed 35.5 $\pm$28.8 % of the dose by the end of the first hour and 52.6 $\pm$21.7 % by the end of the second. In most patients the absorption process was not complete until 4 h after dosing with a mean 65.5 $\pm$18.4 % absorbed. This is in striking contrast to the absorption profile in the normal volunteers where absorption was virtually complete by 90 min (Fig 7.4). However despite this, the total fraction of the dose eventually absorbed was greater in the CAPD patients and the mean AUC to 240 min estimated from the plot of cumulative percent of dose absorbed versus time was 9385 $\pm$4344 and 11025 $\pm$4594 $min.mg.l^{-1}$ in the volunteers and patients respectively. The differences were not statistically significant.

As in the case of the healthy volunteers, there was less variation in the plasma concentrations in the patients on CAPD following the 60 minute intravenous infusion of frusemide than after oral administration (Fig 7.2). The mean C_{max} was 5.95 $\pm$1.58 $mg.l^{-1}$ and the mean $AUC_{0-\infty}$ was 1429 $\pm$425 $min.mg.l^{-1}$. The mean V_d was 16.5 $\pm$8.3 l and was similar to the corresponding value of 14.1 $\pm$3.8 l in the healthy volunteers (Table 7.8).

Table 7.7. Lag time, maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the plasma concentration-time curve ($AUC_{0-\infty}$) bioavailability and half life of elimination ($t_{½}$) of frusemide in 11 patients on continuous ambulatory peritoneal dialysis following the ingestion of 80 mg of frusemide. The bioavailability was calculated as the ratio of the $AUC_{0-\infty}$ after the oral and intravenous administration of the same dose of frusemide on two separate occasions. It could not be calculated accurately in patient 4 due to technical problems on the second day of the study and patient 7 did not receive intravenous frusemide.

No.	Lag* time (min)	Lag** time	C_{max} ($mg.l^{-1}$)	T_{max} (min)	AUC ($min.mg.l^{-1}$)	Bioavail-ability (%)	$t_{½}$ (min)
1	15	15	2.19	120	990	74.7	251.8
2	30	15	3.16	90	1080	64.2	171.4
3	0	13	5.43	60	1937	98.0	226.3
4	30	15	4.68	90	1663		206.9
5	30	15	1.37	180	948	70.8	414.9
6	15	15	4.62	180	1514	78.3	178.8
7	60	30	2.89	240	1112		210.9
8	90	30	1.49	180	469	55.4	134.1
9	0	0	1.18	60	451	50.5	225.7
10	15	15	2.52	120	1254	65.6	290.3
11	0	0	2.45	60	841	73.7	192.0
mean	26	15	2.91	126	1113	70.1	227.6
±sd	26	9	1.37	58	440	13.1	71.3

* calculated from raw data
** calculated from fitted model

Table 7.8. Maximum plasma concentration (C_{max}), area under the plasma concentration time curve ($AUC_{0-\infty}$), volume of distribution (V_d), plasma half life of elimination ($t_{½}$) and total clearance of frusemide in 9 of 11 patients on continuous ambulatory peritoneal dialysis following the intravenous infusion of 80 mg of frusemide over one hour. The data obtained from patient 4 were ommitted due to technical problems and patient 7 did not receive intravenous frusemide.

No.	C_{max} ($mg.l^{-1}$)	AUC ($min.mg.l^{-1}$)	V_d (l)	$t_{½}$ (min)	Total Clearance ($ml.min^{-1}$)
1	7.43	1326	7.3	84.2	60.3
2	7.91	1683	10.2	149.3	47.6
3	5.48	1976	14.6	249.4	40.5
5	4.67	1340	34.6	401.8	59.7
6	7.79	1935	12.4	207.3	41.3
8	4.17	849	19.6	144.3	94.2
9	4.21	892	16.6	128.2	89.7
10	6.03	1913	14.5	240.3	41.8
11	5.13	1142	16.9	166.9	70.1
mean	5.95	1429	16.5	194.9	61.9
±sd	1.58	425	8.3	98.0	20.4

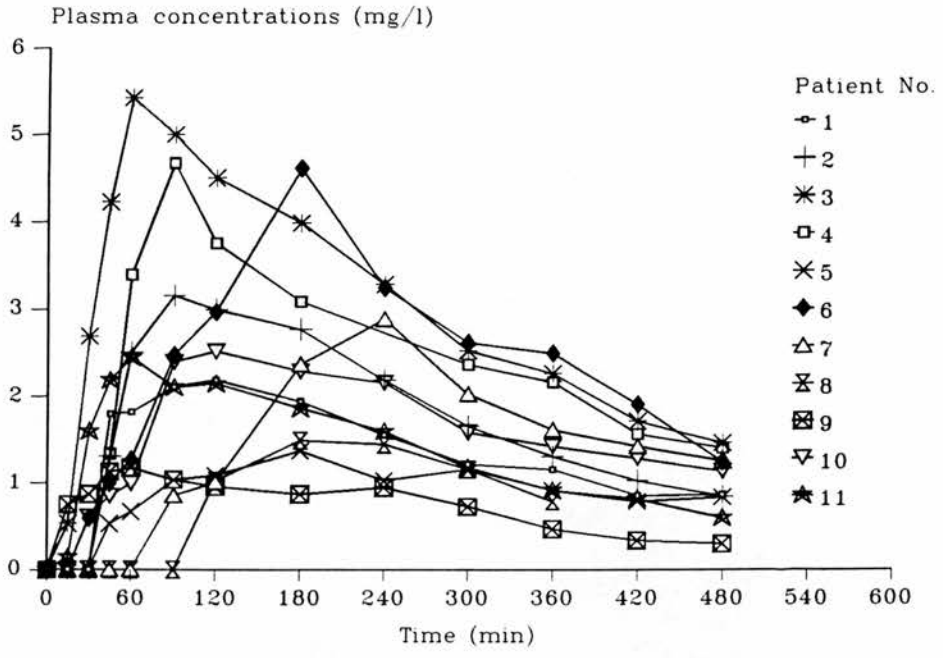


Fig 7.1. Plasma concentrations of frusemide in 11 patients with end stage renal failure maintained on CAPD following the administration of 80 mg orally.

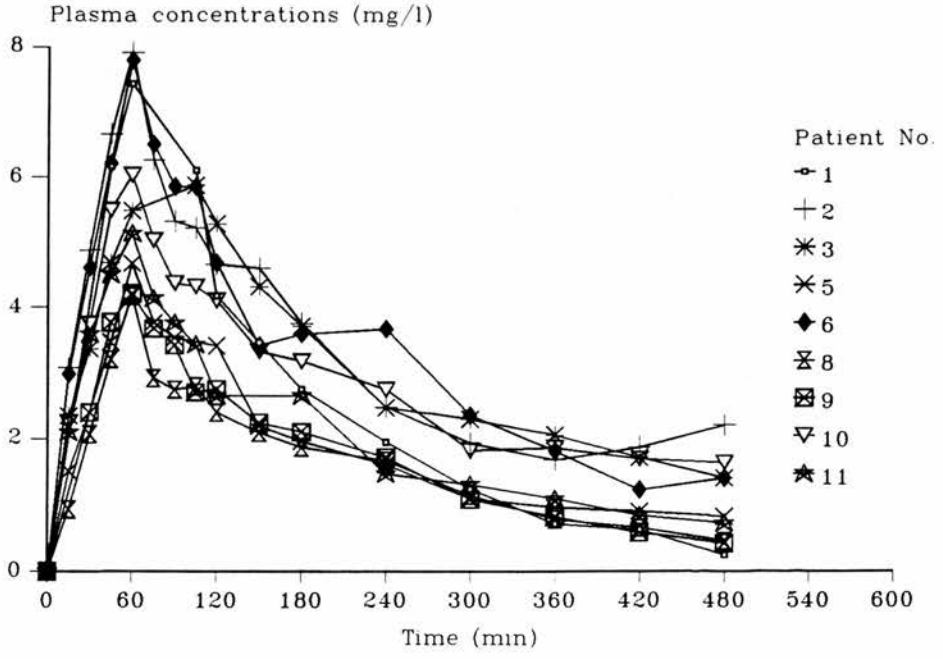


Fig 7.2. Plasma concentrations of frusemide in 9 patients with end stage renal failure maintained on CAPD following the administration of 80 mg by intravenous infusion over 1 h.

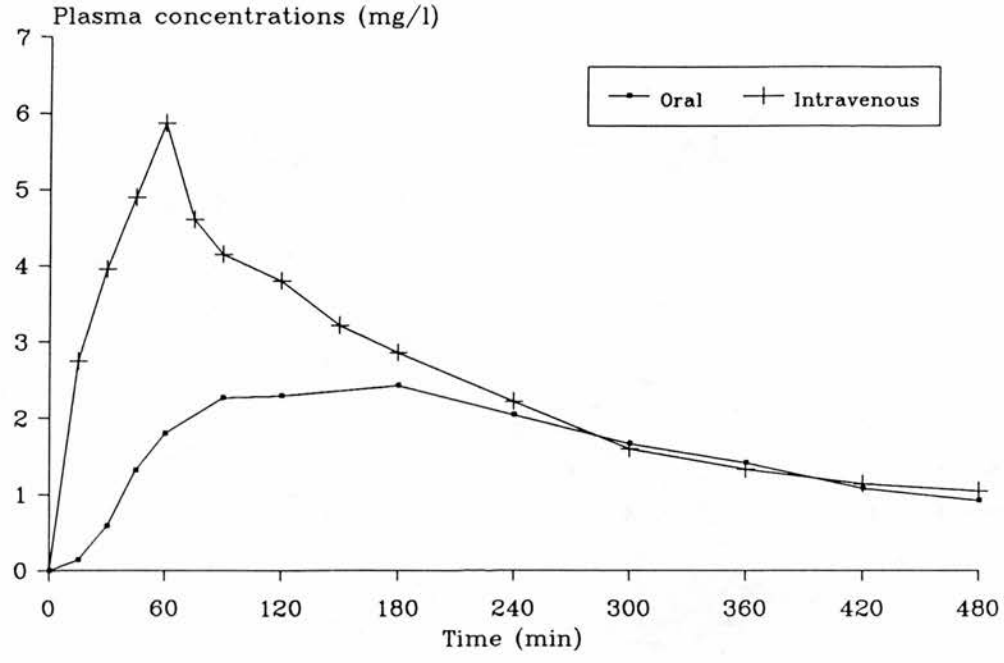


Fig 7.3. The mean plasma concentrations of frusemide in 11 CAPD patients following the oral administration and intravenous infusion (n=9) over 1 h of 80 mg.

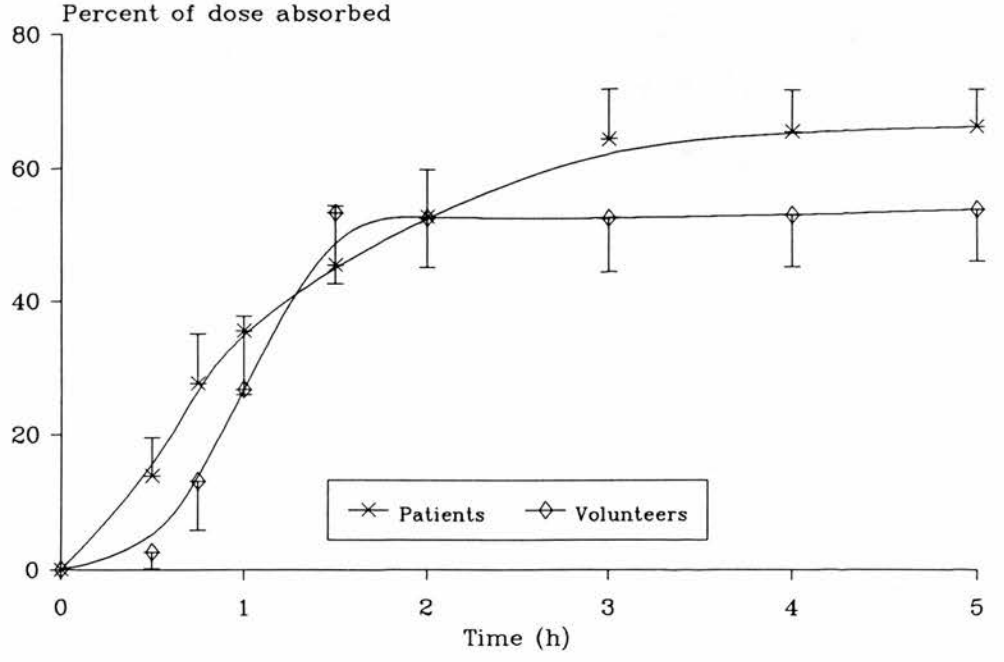


Fig 7.4. The fractional absorption of oral frusemide in CAPD patients compared with healthy volunteers. Results are expressed as mean ±s.e.m.

Elimination of frusemide

The mean elimination half life was 227.6 ±71.3 min following oral frusemide and 194.9 ±98.0 min following the intravenous infusion (Table 7.7 and 7.8). The differences were not statistically significant. The t½ was significantly longer in the patients than in the volunteers where it was 65.1 ±12.0 min ($p<0.001$) and 66.3 ±13.5 min ($p<0.001$) after the oral and intravenous dose respectively. The total clearance of frusemide in the CAPD patients was 61.9 ±20.4 $ml.min^{-1}$ (Table 7.8) and was virtually the same as the non-renal clearance of frusemide in the healthy volunteers (61.4 ±11.8 $ml.min^{-1}$).

Renal excretion of frusemide

Of the 11 patients, 3 were anuric (patients 1,5 and 10). Following the oral dose of 80 mg frusemide, the mean 24 hour urinary volume of the other 8 patients was only 235 ±244 ml (Table 7.9). Very little frusemide was excreted in the urine in 24 hours (0.24 ±0.27 mg or 0.3 ±0.3% of the dose). The mean renal clearance was 0.28 ±0.33 $ml.min^{-1}$ (Table 7.9).

Following intravenous frusemide the mean 24 hour urinary volume was 194 ±242 ml with a mean output of 0.32 ±0.44 mg or 0.4 ±0.6% of the dose over the same period. Three of the patients had no detectable frusemide in the urine (patients 2,4 and 9) and the mean renal clearance was 0.28 ±0.4 $ml.min^{-1}$.

Peritoneal clearance of frusemide

Frusemide was undetectable in all samples of peritoneal fluid. Given that the detection limit of the assay was 0.1 $mg.l^{-1}$ an average sample of 2000 ml dialysate would have to contain about 0.2 mg frusemide in order to give a measurable quantity. In the 24 h period the maximum that could have been excreted in this way would be 0.8 mg or 1 % of the dose of 80 mg. Thus, the peritoneal clearance of frusemide was negligible.

Table 7.9. Twenty four hour urinary volume, urinary recovery and renal clearance of frusemide in 8 of 11 patients on continuous ambulatory peritoneal dialysis given 80 mg frusemide orally and intravenously*. Patients 1, 5 and 10 were anuric.

No.	Urinary volume (ml)	Frusemide recovery (mg)	Renal clearance ($ml.min^{-1}$)
2	76	<0.01	<0.01
2*	58	0.00	0.00
3	160	0.08	0.04
3*	169	0.16	0.08
4	8	0.00	0.00
4*	12	0.00	0.00
6	160	0.30	0.20
6*	197	0.33	0.17
7	505	0.53	0.48
8	185	0.25	0.54
8*	130	0.46	0.54
9	30	0.00	0.00
9*	26	0.00	0.00
11	755	0.78	0.93
11*	763	1.32	1.16
mean	235	0.24	0.28
±sd	244	0.27	0.32
mean*	194	0.32	0.28
±sd*	242	0.44	0.40

SECTION 7.4. DISCUSSION

When frusemide was administered orally to patients with end-stage renal failure its absorption was characterised by marked inter-subject variability. A lag time of 15 to 120 min was observed in 8 of the 11 patients. The mean T_{max} was delayed for more than 2 h compared to 90 min in the volunteers and the absorption process was not complete until 4 h after dosing. However despite this, a greater fraction of the dose was absorbed in the CAPD patients and both the mean bioavailability and the percent absorbed at 4 h were greater than in the volunteers, but not significantly so. Thus, in CAPD patients the absorption of frusemide was slower but tended to be more complete than in the healthy volunteers.

The disposition of frusemide following oral administration in patients with varying degrees of chronic renal failure has been described by several investigators and the findings are summarised on Table 7.10. The results were variable and at times conflicting.

Huang et al., (1975), administered 1 g of frusemide orally to 12 patients with advanced chronic renal failure and found that the T_{max} varied from 2 to 9 h with a mean of 4.4 h. The 4 patients with the most rapid absorption rate had lag times of between 30 and 45 min. Beermann et al., (1977), studied two patients with end-stage renal failure who were not yet on dialysis following the ingestion of 2 and 3 g of frusemide respectively and found that the C_{max} was reached by 3.5 and 3.75 h (Table 7.10).

Riva et al., (1982), studied 7 children with end-stage renal failure undergoing haemodialysis given 75 to 225 mg of frusemide per day. There was no significant difference between the plasma concentrations of frusemide at the time of dosing and 3 h later indicating grossly delayed absorption. In 2 patients given 25 and 75 mg

frusemide, blood was sampled more frequently. In one the T_{max} was 6 h and in the other no peak was observed at all. In contrast, Kühnel et al., (1987), gave repeated doses of 40, 80 and 250 mg of frusemide to patients with moderate chronic renal failure and found the T_{max} was 49.8 $\pm$4.8 min with 40 mg, 86.4 $\pm$54.6 with 80 mg and 58.8 $\pm$27.6 with 250 mg. In some patients however pharmacokinetic analysis was apparently impossible because absorption was markedly delayed and the lag time varied from 8.4 $\pm$6.0 (40 mg) to 15.6 $\pm$0.6 (80 mg) and 21.0 $\pm$15.6 min (250 mg, Table 7.10). There was also a significant decrease in the values of $AUC_{0-\infty}$ and C_{max} normalised for dose following the highest dose suggesting dose-dependent bioavailability.

Finally, Boutron et al., (1981) studied the disposition of frusemide following the administration of 500 and 1000 mg to eleven patients in end-stage renal failure maintained on CAPD. The mean T_{max} was 4 $\pm$1 h with 500 mg and 5 $\pm$2.4 h after 1000 mg (4 patients).

The results of the present study suggest that the absorption of frusemide might be abnormally slow in patients with chronic renal failure and the findings are consistent with previous reports. In 4 previous studies the oral bioavailability of frusemide in patients with chronic renal failure was reported as 75.7 $\pm$19.8% (Huang et al., 1975), 48 $\pm$16 (Kelly et al., 1977), 46.3 $\pm$8.5% (Rane et al., 1978) and 43.4 $\pm$7.4% (Tilstone and Fine, 1978, Table 7.10). The latter three values are somewhat lower than in normal subjects but the present study is in agreement with the report of Huang et al., (1975) in which the bioavailability was higher than in normal volunteers despite the delayed absorption.

The reason why the absorption of frusemide was delayed in these patients with end-stage renal failure is unclear. Little specific information is available on drug absorp-

tion and bioavailability in patients on peritoneal dialysis (Paton et al., 1985). During CAPD the absorption of digoxin (De Paoli Vitali et al., 1981), metronidazole (Bush et al., 1983) and diltiazem (Grech-Bélanger et al., 1988) does not appear to be abnormal. By contrast, the absorption of ketoconazole in CAPD patients was impaired with peak plasma concentrations of only 25% of those achieved with the same dose in normal subjects (Chapman and Warnock, 1983).

It is possible that the gastrointestinal disturbances associated with end-stage renal disease may in some way affect drug absorption in patients on CAPD (Paton et al., 1985). Possible factors include delayed gastric emptying or the effects of other medication. If frusemide is absorbed from the stomach as well as from the intestine as discussed in the previous chapter then delayed gastric emptying and slow gatrointestinal transit might well result in enhanced bioavailability.

All of the patients were taking other medication. Three were receiving ranitidine which may have enhanced the dissociation of frusemide by producing a higher gastric pH and so delayed its absorption. Of the 3 patients, one had no lag time and C_{max} was achieved in 60 min although the bioavailability was only 51%. The other two had lag times of 15 min each, T_{max} values of 2 and 3 h and bioavailabilities of 75 and 78% respectively.

Eight patients were were receiving aluminium hydroxide although they did not take this until one hour after the frusemide. This antacid may alter the absorption of drugs by increasing gastric pH and by delaying gastric emptying but it seems to mostly affect drugs which are highly soluble and rapidly absorbed (Hurwitz et al., 1977). Indeed poorly absorbed drugs may even be absorbed better if intestinal motility is slowed (Manninen et al., 1973).

Five patients were taking metoprolol and ß-adrenergic blockers have been shown to slightly increase the rate of paracetamol absorption by stimulating gastric emptying (Clark et al., 1980). Ten of the 11 patients were on iron therapy and this has been shown to interfer with the absorption of some drugs by forming insoluble chelates (Neuvonen et al., 1970) but this is unlikely to apply to frusemide. Nonetheless, given the wide variety of medication taken by these patients with end-stage renal failure, it is possible that at least some influenced the absorption of frusemide.

Another contributory factor may have been oedema of the gut wall. In a case report of a woman suffering from idiopathic oedema the oral bioavailability of frusemide was less than 20% during an oedematous period compared to 74% in the oedema-free state (Odlind and Beermann, 1980 (b)). Furthermore, in compensated congestive cardiac failure the lag time and T_{max} decreased and the C_{max} of orally administered frusemide increased when compared with the decompensated state (Vasko et al., 1985). In both these situations slow absorption and reduced bioavailability may have been due to gastrointestinal oedema. In the present study, 6 patients had abnormally low plasma albumin concentrations from 28 to 34 $g.l^{-1}$ and this may have contributed to oedema of the gut wall. Finally, it is also possible that alterations in blood flow and permeability during peritoneal dialysis may have delayed drug absorption.

Following the infusion of 80 mg of frusemide over one hour the plasma concentration time data was best described by either a one (three patients) or two (six patients) compartment model. The mean V_d was 16 l and this was similar to that observed in the normal volunteers. The elimination $t_{½}$ was significantly prolonged following both oral and intravenous frusemide compared to the healthy volunteers.

The elimination of frusemide has been studied in patients with various degrees of chronic renal failure and the plasma $t_{½}$ has been reported to vary from normal to as

long as 24 h (Table 7.11). For example, using radiolabelled frusemide, Tilstone and Fine (1978) reported the plasma $t_{½}$ as 14.2 ±2.3 h in patients in end-stage renal failure. The use of radiolabelled material for pharmacokinetic studies of this kind has been criticised however because frusemide metabolites are also measured (Benet, 1979).

Using a fluorometric assay Huang et al., (1975) reported a mean plasma $t_{½}$ of 9.7 h (range 3.6 to 20 h) in a group of conservatively managed patients with advanced renal impairment given 1 g frusemide intravenously. In contrast, Cutler et al., (1974), observed a mean $t_{½}$ of only 80.7 min with a range of 40 to 120 min in patients on haemodialysis. This discrepancy may be explained by the use of a shorter sampling time (Beermann et al., 1977) giving a spuriously low $t_{½}$ (Chennavasin et al., 1981). Finally, in CAPD patients the plasma half life of frusemide after an oral dose of 500 mg was reported 10.5 ±1.2 h (Boutron et al., 1981, Table 7.11).

Riva et al., (1982) and Keller et al., (1981) studied frusemide disposition in 2 children on haemodialysis and a group of adults on intermittent peritoneal dialysis respectively using gas liquid chromatography. Similar $t_{½}$ values of 3.7 and 4.5 h were found in the children and the corresponding value in the adults was 3.3 ±0.9 h (Table 7.11).

HPLC has often been used to measure frusemide (Table 7.11). Voelker et al., (1987) studied the disposition of frusemide in conservatively managed patients with chronic renal failure (creatinine clearance 3-27 $ml.min^{-1}$) and reported the mean $t_{½}$ as 2.6 ±0.3 h. The same value of 2.6 ±0.4 $ml.min^{-1}$ was found in a similar group of patients and it was not altered by haemodialysis (Rane et al., 1978). In a separate group (creatinine clearance 0.6-53 $ml.min^{-1}$) the $t_{½}$ varied from almost normal up to 24 h and it was unrelated to the degree of renal impairment (Beermann et al., (1977).

In most reports the elimination half life of frusemide in patients with various degrees of renal impairment is in the range of 2 to 4 h and this is in good agreement with the present study. However, both shorter and much longer values have been reported not consistently related to the assay, route of administration or the dose of frusemide. The problem may be compounded by the different methods of curve fitting as discussed for the normal volunteer studies (Chennavasin et al., 1981).

In patients with end-stage renal failure, frusemide was cleared essentially by non-renal mechanisms and renal contribution was negligible. The former route accounts for about half the plasma clearance of frusemide in normal volunteers so the value of 62 $ml.min^{-1}$ obtained in the present study is consistent with this. However, the values reported by others for the non-renal clearance of frusemide vary greatly and a reduction has been noted in patients with chronic renal failure (Rane et al., 1978, Beermann et al., 1977).

Less than 1 % of the dose was eliminated by peritoneal dialysis and the removal of frusemide by this route was negligible. Similar findings were reported by Boutron et al., (1981) who found that less than 1% of an oral dose of 500 or 1000 mg was excreted in the dialysate in CAPD patients. Although peritoneal dialysis is an effective method for removing uraemic waste products it contributes little to the clearance of most drugs (Paton et al., 1985). The low flow rate of the peritoneal effluent equivalent to approximately 10 litres per day or 7 $ml.min^{-1}$ limits the clearance of the peritoneal membrane (Keller et al., 1990). Removal of drugs during dialysis needs to be considered clinically only if the total clearance increases by 30% or more (Lee and Marbury, 1984). When drugs are eliminated to a significant extent by non-renal mechanisms the contribution of CAPD to their clearance is minimal (Keller et al., 1990). This certainly applies to frusemide.

The pharmacokinetic properties of a drug which influence its removal from the body during peritoneal dialysis are its volume of distribution, the degree to which it is bound to plasma proteins and its non-renal clearance (Paton et al., 1985). In order for drugs to be significantly eliminated by CAPD they need to have a low volume of distribution to provide high plasma concentrations and hence an effective concentration gradient to diffuse in the peritoneal cavity to an appreciable extent. The other important factor is the extent of protein binding (Peterson and Gerding, 1980). Although the plasma protein binding of frusemide is reduced in uraemia it is still 94 to 97% (Rane et al., 1978, Andreason et al., 1978). In the present study the plasma albumin concentrations were below normal in 6 of the patients and thus the free fraction of frusemide may have been increased slightly. Nonetheless, the fraction of bound frusemide was undoutedly still very high which would have limited the amount of frusemide removed from the body by CAPD despite the low volume of distribution of the drug. Thus, because of the high plasma protein of frusemide and the significant clearance of the drug by non-renal mechanisms, the low peritoneal clearance was not unexpected.

The present findings indicate as in other reports that the absorption of frusemide was markedly delayed in patients with end-stage renal failure compared to healthy volunteers. Despite this the total amount of frusemide absorbed tended to be higher in these patients than in the volunteers possibly due in part to uraemia and interactions with other medication. Frusemide was eliminated almost completely by non-renal mechanisms but little was cleared across the peritoneal membrane possibly due to high plasma protein binding.

Table 7.10. Pharmacokinetic variables of oral frusemide administered to patients with varying degrees of chronic renal failure

Author & year	Dose (mg)	Assay	No.	Renal status Cr Cl ($ml.min^{-1}$)	T_{max} (mins)	Bioavail-ability (%)	lag time (mins)	F_{ex} (%)
Beermann	2000	GLC	2	3	210			1.4
1977	3000	HPLC			228			0.7
Boutron	500	Fl	11	end-stage	240 ±60			4.7 ±1.1
1981	1000				330 ±138			5.8 ±0.9
Huang	1000	Fl	12	1420 ±569*	264	76 ±20		
1974					(120-540)			
Kelly	240	^{35}S	9	4-77	(100-120)	48 ±16		
1977								
Kuhnel	40 x4	Fl	12	350 ±295*	50 ±5		8 ±1	
1987	80 x4				86 ±55		16 ±6	
	120 x4				59 ±28		21 ±16	
Rane	80	HPLC	6	11 ±1		46 ±9		
1978								
Riva	25,75	GLC	2		360			
1982								
Tilstone	500	^{35}S	13	<5		43 ±7		
1978								

*creatinine ($\mu mol.l^{-1}$), Fl = fluorometric assay, GLC = gas liquid chromatography, ^{35}S = radiolabelled frusemide
Cr Cl = creatinine clearance, F_{ex} = percent of dose excreted in urine

Table 7.11. Pharmacokinetic variables in patients with renal failure following single doses of intravenous frusemide

Author & year	Dose (mg)	Assay	No.	Renal status Cr Cl ($ml.min^{-1}$)	$t_{½}$ (hr)	Cl_p ($ml.min^{-1}$)	Cl_r ($ml.min^{-1}$)	Cl_{nr} ($ml.min^{-1}$)	F_{ex} (%)	V_d (l)
Andreasen 1978	40	Fl TLC	7	end-stage HD	1.9 ±0.6	66 ±19	-	66 ±19		10.0 ±2.5
Beermann 1977	40-490	GLC HPLC	14	1-53	1.2 -24.6	77 ±27 (n=5)	9 ±16 (n=5)	28 ±39		
Brater 1986	120-160	HPLC	8	5-18					11 ±2	
Cutler 1974	120	Fl	5	end-stage HD	1.4 ±0.3	104 ±29	-	104 ±29		179 ±38**
Huang 1974	1000	Fl	12	1420 ±569*	9.7 ±4.0	38 ±5 (n=7)	3 ±2 (n=7)	36 ±6 (n=7)		327 ±98**
Keller 1981	40	GLC	7	end-stage IPD 0-3.7	3.3 ±0.9	56 ±28	1-2 (n=3)			14.4 ±3.6
Kelly 1977	240	^{35}S	6	4-77	1.2 ±0.3	92 ±36	42 ±13	50 ±42		14.1 ±5.8
Rane 1978	80	HPLC	6	11 ±1	2.6 ±0.4	51 ±7	5 ±1	46 ±7		124 ±9
Riva 1982	1mg.kg^{-1}	GLC	2	<10	3.7,4.5	12,15	3	10		3.6,3.8
Rose 1976		^{14}C	10	14-96			17-133			
Tilstone 1978	22	^{35}S	13	<5	14.2 ±2.3	15		15		367 ±63**
Traeger 1984	40, 80	Fl	18	100-250*	1.5 ±0.2	64 ±13	23 ±6	41 ±11	30	5.2 ±1.1
				250-1150	1.8 ±0.3	60 ±13	5 ±0.9	55 ±13	8	
Voelker 1987	80-600	HPLC	10	3-27	2.6 ±0.3	59 ±5	6 ±1	53 ±5	9 ±2	

*creatinine ($\mu mol.l^{-1}$), **$ml.kg^{-1}$, Fl = fluorometric, GLC = gas liquid chromatography, TLC = thin-layer chromatography, ^{35}S & ^{14}C = radiolabelled frusemide, HD = haemodialysis, IPD = intermittant peritoneal dialysis, Cr Cl = creatinine clearance, $Cl_{p,r,nr}$ = plasma, renal and non-renal clearance, F_{ex} = % dose excreted in urine

CHAPTER 8

THE DISPOSITION OF FRUSEMIDE DURING LONG TERM THERAPY IN CONSERVATIVELY MANAGED PATIENTS WITH CHRONIC RENAL FAILURE

SECTION 8.1: INTRODUCTION

Frusemide is extensively used in patients with chronic renal failure and it remains an effective diuretic even when renal function is severely impaired (Allison and Kennedy, 1971). Frusemide must gain access to the tubular lumen to exert an effect (Chennavasin et al., 1979), and its active secretion may be blocked by endogenous organic acids in chronic renal failure (Rose et al., 1976). This may necessitate the use of very large doses (Brater et al., 1986). With the use of such doses chronically, it is possible that accumulation of frusemide might occur in the plasma due to reduced renal elimination. Although most of the side effects of frusemide including hyponatraemia and hypokalaemia relate to its effects on the kidney, plasma concentrations of over 50 $mg.l^{-1}$ have been associated with ototoxicity (Lloyd Mostyn and Lord, 1971, Morelli et al., 1971, Gallagher and Jones, 1979).

In healthy volunteers the non-renal clearance of frusemide accounts for about 50 % of the total clearance and in CAPD patients it was eliminated almost entirely by non-renal mechanisms. It seems likely that conservatively managed patients will also depend to a significant degree on non-renal clearance to eliminate the large daily doses of frusemide which they often require. The site of non-renal clearance is unclear however (Branch, 1983) and a reduction has been noted in patients with uraemia (Rane et al., 1978).

Although the disposition of frusemide has been studied extensively in patients with chronic renal failure following a single dose, the results are often conflicting and little attention has been given to patients taking the diuretic chronically in large doses. The purpose of this study was to investigate the handling of frusemide in such patients in order to assess the degree of accumulation during chronic dosing. It was hoped to elucidate the absorption of frusemide under these circumstances and to determine the

amount which is delivered to its active site in the kidney and to see how closely this relates to the degree of renal impairment. Finally, it was of interest to investigate the elimination of frusemide during chronic administration of large doses in order to assess the adequacy of non-renal clearance in such patients with impaired renal function.

SECTION 8.2: METHODS

Patients

Ten patients (5 male, 5 female) on conservative management for stable chronic renal failure took part in the study. Their mean age was 52 yr (range 32 - 68) and mean weight 76 kg (range 66 - 88). Details of the medical histories and medication are presented in Table 8.1 and results of haematology and biochemistry screening in Tables 8.2, 8.3 and 8.4. The mean plasma creatinine was 516 $\pm$268 μmol.l^{-1} and mean creatinine clearance 21 $\pm$17 ml.min^{-1} (Table 8.3). Although 4 of the patients had proteinuria in the nephrotic range (>4.5 g.l^{-1}) only patient 3 was hypoalbuminaemic. The other 3 had plasma albumin concentrations at the lower limit of normal (Table 8.2). All the patients were on once daily maintenance doses of frusemide (Hoechst, U.K. Ltd.,) ranging from 80 to 500 mg (Table 8.1) and renal function had remained stable in the 3 months before the study. Patients were encouraged to maintain a high fluid intake of 2 to 3 l daily except for patient 4 who was restricted to a daily fluid intake of 500 ml.

Experimental design

The patients were studied on 2 separate occasions which were usually on consecutive days. For one week before the study the patients were instructed to take the usual maintenance dose of frusemide at exactly the same time each morning 1 h before breakfast. In the 48 h before the study the patients made two 24 h collections of urine.

On the morning of the first study day the fasting patients attended at the Clinical Pharmacology Unit at 08.30 h and a cannula was inserted into a forearm vein. The patients were then asked to take their normal dose of frusemide and other medications (Table 8.1) with 200 ml of water, after which they remained recumbent for 1 h.

Breakfast was served at 09.30 h and lunch at 13.00 h according to the patient's normal low protein, low salt dietary requirements. The patients were allowed their normal daily fluid allowance taken as water or still orange juice and they regulated this themselves throughout the day.

Blood (3 ml) was sampled before and then at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after the frusemide was given and urine was collected at 0, 30, 60, 120, 240, 360 and 480 min. Patients were free to go at 16.30 h and were asked to collect all urine passed until the end of the 24 h period after the administration of frusemide.

On the second study day the procedure was the same but half of the usual maintenance dose of frusemide was infused intravenously over 1 h at a constant rate in 100 ml of 0.9% saline via an "IMED 960" volumetric infusion pump. The patients took their regular medications at 08.30 h. Sampling was the same as described above with extra blood samples at 7.5, 105 and 150 min after the start of the infusion.

Samples

All samples were processed as described in Chapter 2.

Drug Assay

Plasma and urinary concentrations of frusemide were measured by HPLC as described in Chapter 2.

Table 8.1. Clinical details of 10 patients on conservative management for chronic renal failure.

Patient No.	Age & sex	Medical diagnoses	Regular drug therapy	Frusemide (mg)
1	62 F	pyelonephritis hypertension	nifedipine 20 mg TID metoprolol 100 mg TID alfacalcidol 0.5 μg daily aluminium hydroxide 475 mg TID	80
2	34 M	glomerulonephrits hypertension	metoprolol 50 mg TID enalapril 10 mg daily sodium bicarbonate 300 mg TID ranitidine 75 mg nocte	250
3	54 M	glomerulonephritis hypertension	allopurinol 100mg daily captopril 50 mg TID	160
4	68 F	?analgesic nephropathy ischaemic heart disease hypertension	captopril 50 mg TID isosorbide mononitrate 25 mg TID	240
5	41 F	S.L.E. hypertension cardiac failure	captopril 12.5 mg TID digoxin 0.0625 mg mane warfarin 5 mg nocte prazosin 1 mg TID prednisolone 15 mg daily azothioprine 75 mg daily chloroquine 125 mg nocte	250
6	54 F	polycystic kidneys hypertension	captopril 50 mg BD nifedipine slow release 20 mg TID aluminium hydroxide 475 mg nocte	160
7	51 M	Wegner's granulo -matosus	cyclophosphamide 50 mg daily prednisolone 12.5 mg ranitidine 75 mg BD	120
8	57 M	glomerulonephritis hypertension	captopril 50 TID nifedipine 20 mg BD allopurinol 100 mg daily thyroxine 500 μg daily metoprolol 50 mg BD	250
9	51 M	glomerulonephritis hypertension	enalapril 10 mg daily nifedipine 20 mg BD metolozone 5 mg daily metoprolol 25 mg BD	500
10	44 M	polycystic kidneys urinary tract infection hypertension	atenolol 100 mg daily "slow K" TID cephalexin 150 mg nocte	80

S.L.E. = systemic lupus erythematosus

Table 8:2. Clinical biochemical test results in 10 patients with chronic renal failure taking regular frusemide therapy.

	Plasma concentration								
Patient No.	Protein $g.l^{-1}$	Albumin $g.l^{-1}$	Calcium $mmol.l^{-1}$	Phosphate $mmol.l^{-1}$	Alkaline phosphatase $u.l^{-1}$	Bilirubin $\mu mol.l^{-1}$	ALT $u.l^{-1}$	Ggt $u.l^{-1}$	Bicarbonate $mmol.l^{-1}$
1	69	45	2.4	1.8	63	5	21	15	25
2	63	37	2.4	1.6	94	7	14	19	24
3	54	32	2.2	1.3	84	4	16	22	26
4	67	42	2.2	2.0	93	4	7	10	14
5	66	42	2.1	1.4	48	6	21	13	21
6	67	44	2.3	1.8	91	4	18	12	22
7	66	43	2.4	1.1	60	6	10	30	23
8	64	38	2.2	1.2	132	4	30	12	24
9	64	37	2.2	1.9	71	5	15	48	16
10	66	41	2.4	1.3	65	3	10	12	25
Normal range	60-80	36-47	2.1-2.6	0.8-1.4	40-100	2-17	10-40	10-55	24-30

Table 8:3. Renal function tests in 10 patients with chronic renal failure taking regular frusemide therapy.

Patient No.	Plasma Creatinine $\mu mol.l^{-1}$	Plasma urea $mmol.l^{-1}$	Creatinine clearance $ml.min^{-1}$	Urinary protein $g.24h^{-1}$
1	607	26.8	13	0.3
2	647	25.5	13	5.7
3	581	23.6	16	4.6
4	337	33.2	8	0.4
5	457	26.0	23	1.0
6	777	25.6	6	0.8
7	354	14.3	18	2.7
8	222	11.4	48	7.7
9	1071	42.8	7	5.1
10	112	9.3	56	0.3
Normal range	55-150	2.5-6.6	>100	<0.1

Table 8.4. Results of haematological tests in 10 conservatively managed patients with chronic renal failure taking regular frusemide therapy.

Patient	Haemoglobin concentration $g.l^{-1}$	white cell count $\times 10^9.l^{-1}$	platelets $\times 10^9.l^{-1}$
1	11.7	5.2	234
2	8.3	6.3	250
3	9.5	9.1	282
4	10.7	8.3	172
5	10.3	8.9	254
6	9.8	4.5	291
7	10.4	5.9	216
8	15.0	8.0	264
9	9.0	8.5	221
10	15.2	8.3	231
Normal range	13-18	4-11	150-350

Pharmacokinetic analysis

When blood samples contained residual amounts of frusemide from the previous dose, corrected plasma concentrations were calculated using Equation 2.1 as described in Chapter 2. The SIPHAR pharmacokinetic programme was then used to analyse the corrected data as described in Chapter 2.

The plasma concentration-time curves obtained with oral dosing were fitted to a one compartment model. Initial parameters were estimated by peeling and refined by iterative analysis. The goodness of the fit was established by comparing the coefficients of variation of each parameter as described in Chapter 2 and these are listed in Table 8.5. They were usually less than 20-30 % except for patients 3, 5 and 6 where they were slightly greater.

Following the intravenous dose the plasma concentration-time curves were best described by a two compartment model. Coefficients of variation of the estimated parameters were less than 20-30% in all cases except for patient 2 (Table 8.6).

The fitted model was used to describe the disposition of oral and intravenous frusemide and to estimate the lag time and the elimination half life ($t_{½}$). The Wagner-Nelson method was used to estimate the percent of the dose absorbed with time as described in Chapter 2. Model independent analysis was used to estimate the $AUC_{0-\infty}$, the total, renal and non-renal clearances and the V_d.

Statistics

The significance of observed differences was determined using the Students "t" test or the Wilcoxon or Mann-Whitney test for paired and un-paired data respectively, where appropriate.

Table 8.5. The coefficients of variation (CV%) for the estimated parameters obtained by analysis of the plasma concentration time data following the administration of frusemide 80 to 500 mg orally to conservatively managed patients with chronic renal failure. The data were best described by a one compartment model comprising an absorption and elimination phase. Initial parameters were estimated by peeling and refined by iterative analysis. The absorption phase is represented by intercept "A" and slope "α" and the elimination phase by intercept "B" and slope "ß" and described in Chapter 2.

	Absorption phase		Elimination phase	
No.	Intercept "A" (CV%)	Slope "α" (CV%)	Intercept "B" (CV%)	Slope "ß" (CV%)
1	0.0	9.4	0.0	21.2
2	1.2	14.9	2.7	14.0
3	44.0	33.1	48.8	31.8
4	14.5	24.3	23.2	26.0
5	34.8	35.0	55.0	52.1
6	36.1	31.9	42.0	33.1
7	14.5	18.1	20.2	15.4
8	11.2	15.2	13.1	9.5
9	12.6	21.3	20.6	16.3
10	15.1	18.0	26.1	10.8

Table 8.6. The coefficients of variation (CV%) for the estimated parameters obtained by analysis of the plasma concentration time data obtained following the administration of frusemide 40 to 250 mg intravenously to conservatively managed patients with chronic renal failure.The data were best described by a two compartment model comprising a distribution and elimination phase. Initial parameters were estimated by peeling and refined by iterative analysis. The distribution phase is represented by intercept "D" and slope "d" and the elimination phase by intercept "B" and slope "ß" as described in Chapter 2.

	Distribution phase		Elimination phase	
No.	Intercept "D" (CV%)	Slope "d" (CV%)	Intercept "B" (CV%)	Slope "ß" (CV%)
1	11.9	26.6	11.0	18.3
2	28.2	42.8	6.0	6.3
3	9.2	18.8	17.9	28.9
4	15.1	26.1	2.6	2.1
5	6.4	14.6	5.0	4.2
6	7.6	17.4	6.5	5.5
7	7.1	13.0	7.3	4.0
8	4.8	9.4	4.8	2.7
9	9.0	21.3	6.9	4.4
10	13.0	6.6	28.1	15.5

SECTION 8.3: RESULTS

Plasma concentrations of frusemide

The individual plasma concentration-time curves following the oral and intravenous administration of frusemide are shown in Figures 8.1 and 8.2 respectively. Plasma concentrations were corrected for residual traces of frusemide which was present in all baseline samples (C_0) except patient 8, study day 2. The values varied from 0.12 $mg.l^{-1}$ to 1.07 $mg.l^{-1}$ (Tables 8.7 and 8.8).

The absorption and distribution of frusemide

The oral absorption of frusemide in conservatively managed patients with chronic renal failure was characterised by marked inter-subject variability (Fig. 8.1). The patients were all taking different doses of frusemide however which may have accounted for some of this variability. In most cases frusemide was absorbed rapidly with a mean lag time derived from the raw data of 5 $\pm$7 min and in 7 of the 10 patients no lag time was observed. Using the fitted model, the mean lag time was longer at 13 $\pm$9 min (Table 8.7).

The mean C_{max} following the oral administration of frusemide to these patients was 7.29 $\pm$4.34 $mg.l^{-1}$ and the mean T_{max} was 102 $\pm$46 min (Table 8.7). As expected the $AUC_{0-\infty}$ was variable with a mean value of 2570 $\pm$1461 $min.mg.l^{-1}$. The mean bioavailability of oral frusemide was 61.3 $\pm$18.8 % (Table 8.7). The corresponding values for T_{max} and bioavailability did not differ significantly in the healthy volunteers (90 $\pm$16 min and 53.6 $\pm$21.3 % respectively, Table 8.9).

Following the oral administration of frusemide in the CAPD patients the lag time derived from the raw data of 26 $\pm$26 min was significantly longer ($p < 0.05$) than the corresponding value of 5 $\pm$7 min observed in the conservatively managed group.

However, when the fitted model was used the lag times were similar in both groups of patients at 15 ±9 and 13 ±9 min, respectively. Similarly, although the T_{max} was longer in the CAPD patients at 126 ±58 min and the bioavailability higher at 70.1 ±13.1 %, the differences failed to reach statistical significance (Table 8.9).

The fraction of the dose absorbed with time was also compared (Figs 8.3 and 8.4). In the conservatively managed patients 42.0 ±22.8 % of the dose was absorbed in 1 h. The corresponding percentages in the healthy volunteers and CAPD patients were less but not significantly so at 26.7 ±28.8 and 35.5 ±28.8 % respectively. By the end of the second h 54.1 ±18.6 % of the dose had been absorbed by the conservatively managed patients and similar amounts had been absorbed by the volunteers and CAPD patients (52.4 ±19.6 and 52.6 ±21.7 %, respectively). The absorption process was similar in the healthy volunteers and conservatively managed patients with little further absorption after 2 h (Fig 8.3). In contrast, the absorption process was not complete in the CAPD patients for 4 h (Fig 8.4). Despite this, the cumulative AUC to 4 h calculated from the plot of the percent of the dose absorbed with time was not significantly different in the healthy volunteers (9385 ±4344 $min.mg.l^{1}$), CAPD patients (11025 ±4594 $min.mg.l^{-1}$) and the conservatively managed patients with chronic renal failure (10956 ±3957 $min.mg.l^{-1}$).

Following the intravenous infusion of frusemide the mean C_{max} in the conservatively managed patients was 9.24 ±4.70 $mg.l^{-1}$ (Table 8.8). The V_d of 12.8 ±2.1 l was similar to that observed in the healthy volunteers (14.1 ±3.8 l). The V_d in the CAPD patients was slightly higher at 16.5 ±8.3 l but the differences were not significantly different (Table 8.9).

Table 8.7. Lag time, residual plasma concentration at time zero (C_0), maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), bioavailability and half life of elimination ($t_{½}$) of frusemide in 10 conservatively managed patients with chronic renal failure following the ingestion of 80 to 500 mg of frusemide. The bioavailability was calculated as the ratio of the $AUC_{0-\infty}$ after the oral and intravenous administration of frusemide on two separate occasions.

No.	Lag* time (min)	Lag** time (min)	C_0 ($mg.l^{-1}$)	C_{max} ($mg.l^{-1}$)	T_{max} (min)	AUC ($min.mg.l^{-1}$)	Bioavail-ability (%)	$t_{½}$ (min)
1	0	0	0.74	5.71	45	2627	87.0	371.6
2	15	15	1.06	4.29	180	2645	43.3	324.2
3	0	8	0.44	4.24	120	1520	40.5	177.7
4	0	14	0.45	6.44	120	2790	60.4	250.4
5	0	0	0.99	8.75	180	3985	74.5	262.1
6	0	10	0.57	6.25	90	1666	41.0	202.3
7	15	24	0.12	7.61	90	1991	93.1	154.8
8	0	13	0.37	9.20	45	2250	62.0	140.9
9	0	14	0.20	18.67	90	5971	70.8	174.6
10	15	28	0.64	1.77	60	252	40.4	76.7
mean	5	13	0.56	7.29	102	2570	61.3	213.5
±sd	7	9	0.29	4.34	46	1461	18.8	84.5

* calculated from raw data
** calculated from fitted model

Table 8.8. Residual plasma concentration at time zero (C_0), maximum plasma concentration (C_{max}), area under the plasma concentration time curve ($AUC_{0-\infty}$), volume of distribution (V_d), plasma half life of elimination ($t_{½}$) and total clearance of frusemide in 10 conservatively managed patients with chronic renal failure following the intravenous infusion of 40 to 250 mg of frusemide over one hour.

No.	C_0	C_{max}	AUC	V_d	$t_{½}$	Total Clearance
	($mg.l^{-1}$)		($min.mg.l^{-1}$)	(l)	(min)	($ml.min^{-1}$)
1	1.04	3.81	1509	13.3	347.7	26.5
2	1.07	8.70	3054	11.7	198.7	40.9
3	0.57	5.61	1878	16.5	358.9	31.9
4	0.88	11.04	2308	11.4	152.5	52.0
5	0.84	10.86	2673	13.0	192.8	46.8
6	0.53	9.49	2029	10.3	181.3	39.4
7	0.40	6.35	1069	11.0	136.0	56.1
8	0.00	12.36	1815	11.6	117.0	68.9
9	0.16	20.40	4219	12.1	141.7	59.3
10	0.72	3.80	312	16.7	90.3	128.0
mean	0.62	9.24	2087	12.7	191.7	55.0
±sd	0.34	4.70	1025	2.1	86.9	27.2

Table 8.10. Comparison of pharmocokinetic variables in healthy volunteers, conservatively managed patients with chronic renal failure and CAPD patients following the administration of frusemide orally and intravenously (iv).

	Healthy volunteers	Conservatively managed patients	CAPD patients
T_{max} (min)	90 ±16	102 ±46	126 ±58
Bioavailability (%)	53.6 ±21.3	61.3 ±18.8	70.1 ±13.1
V_d (l)	14.1 ± 3.8	12.7 ± 2.1	16.5 ± 8.3
$t_{\frac{1}{2}}$ (oral) (min)	65.1 ±12.0	213.5 ±84.5	227.6 ±71.3
$t_{\frac{1}{2}}$ (iv) (min)	66.3 ±13.5	191.7 ±86.9	194.9 ±98.0
Total clearance ($ml.min^{-1}$)	137.8 ±26.9	55.0 ±27.2	61.9 ±20.4
Renal clearance (oral)	93.2 ±33.6	11.8 ±21.6	0.3 ± 0.3
Renal clearance (iv)	76.5 ±18.0	11.2 ±19.4	0.3 ± 0.4
f_{ex} (oral) (%)	34.7 ± 8.9	7.4 ± 5.4	0.3 ± 0.3
f_{ex} (iv) (%)	55.2 ± 4.8	7.9 ± 6.3	0.4 ± 0.6
Non-renal clearance ($ml.min^{-1}$)	61.4 ±11.8	43.8 ±12.2	61.9 ±20.4

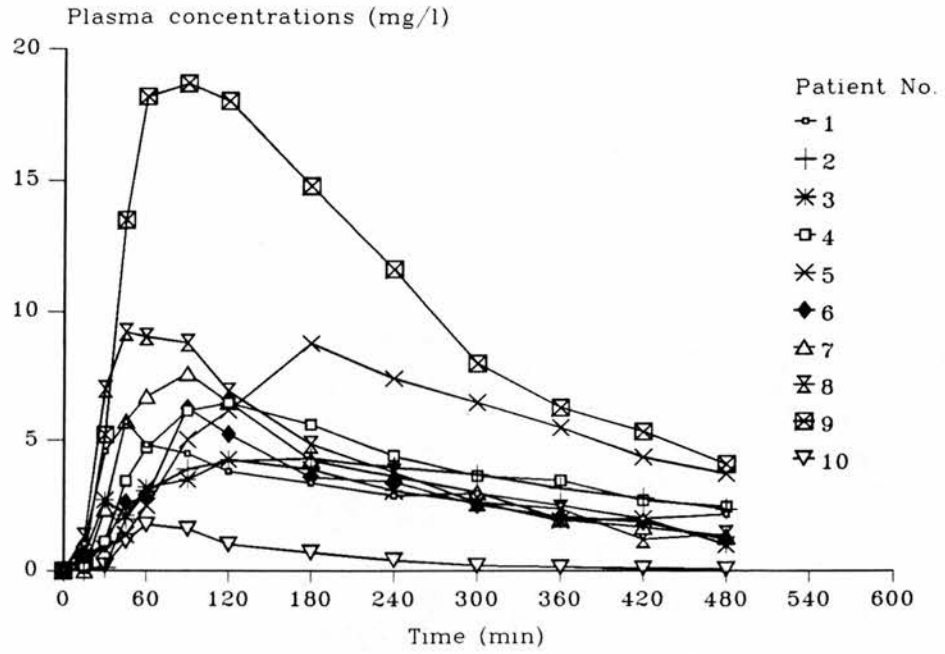


Fig 8.1. Plasma concentrations of frusemide in 10 conservatively managed patients with chronic renal failure following the administration of 80 to 500 mg orally.

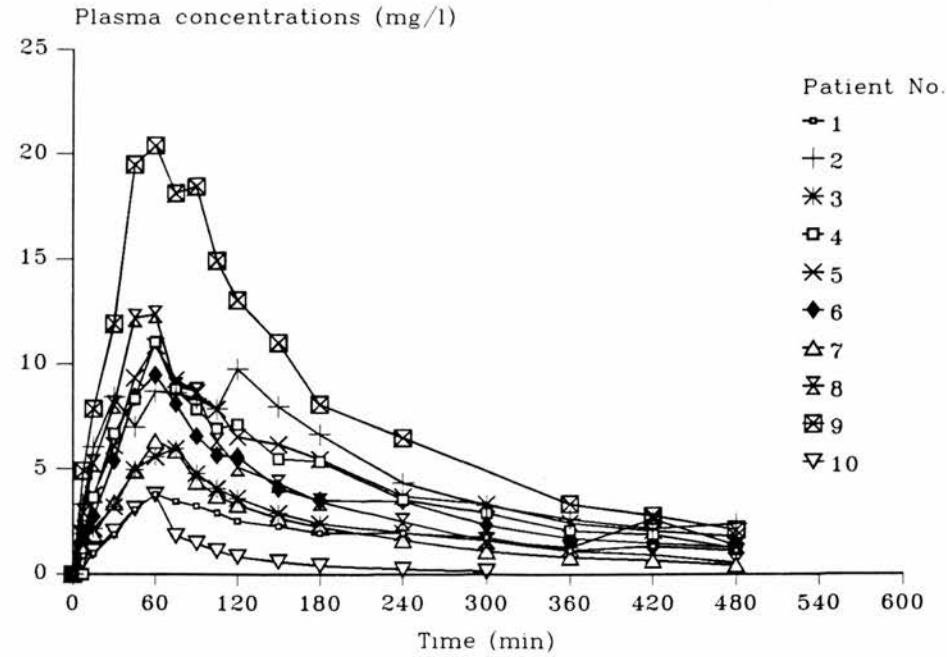


Fig 8.2. Plasma concentrations of frusemide in 10 conservatively managed patients with chronic renal failure following the administration of 40 to 250 mg by intravenous infusion over 1 h.

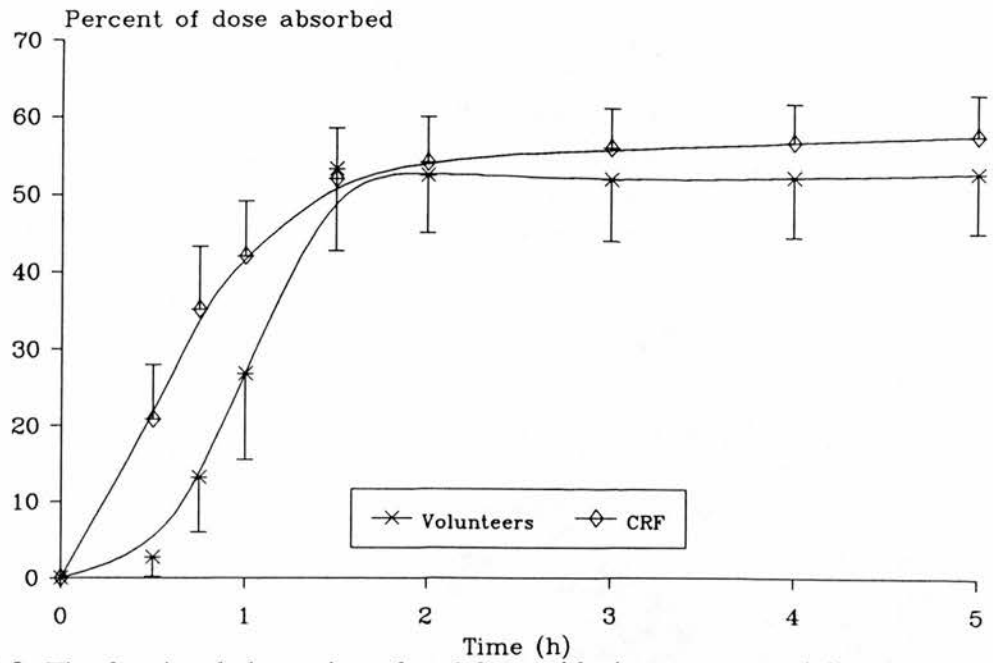


Fig 8.3. The fractional absorption of oral frusemide (mean ±s.e.m.) in conservatively managed patients with chronic renal failure compared with healthy volunteers.

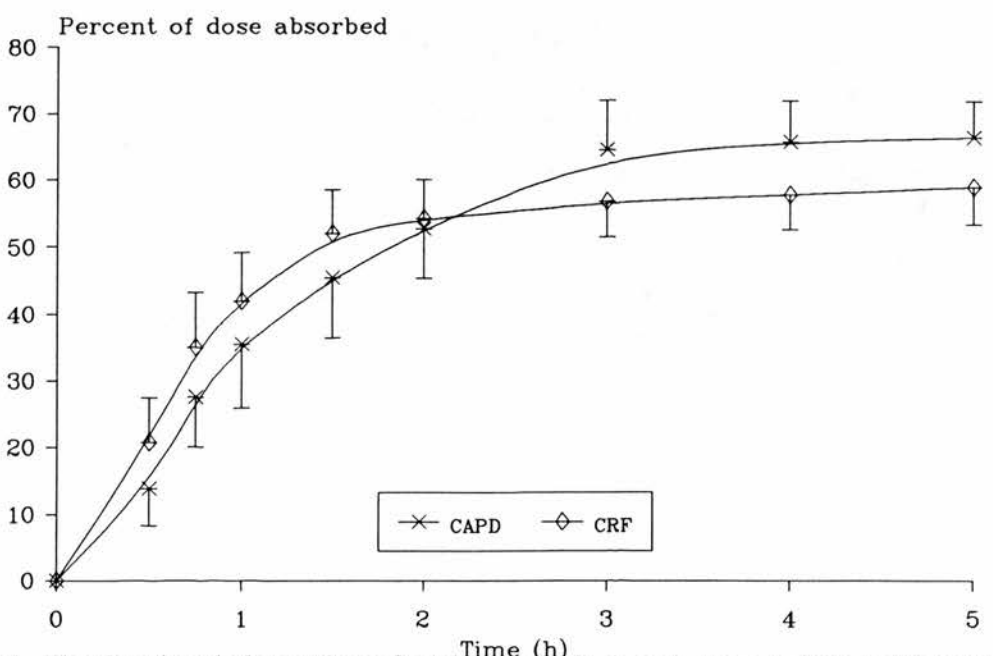


Fig 8.4. The fractional absorption of oral frusemide (mean ±s.e.m.) in conservatively managed patients compared with CAPD patients.

Elimination of frusemide

In patients with chronic renal failure the mean elimination half life of frusemide was 213.5 ±84.5 and 191.7 ±86.9 min respectively following oral and intravenous dosing (Tables 8.7 and 8.8). The differences between routes were not statistically significant. The t½ was significantly longer ($p < 0.001$) following both routes of administration than the corresponding values of 65.1 ±12.0 and 66.3 ±13.5 min respectively observed in the healthy volunteers. However, the t½ values in the patients with chronic renal failure are remarkably similar to those observed in the CAPD patients (227.6 ±71.3 and 194.9 ±98.0 min) following the oral and intravenous routes of administration (Table 8.9). The total body clearance of intravenous frusemide was 55.0 ±27.2 $ml.min^{-1}$ and this was significantly less than the corresponding value of 137.8 ±26.9 $ml.min^{-1}$ observed in the volunteers ($p < 0.001$, Tables 8.8 and 8.9).

Renal excretion of frusemide

When oral frusemide was administered daily to patients with chronic renal failure in doses of 80 to 500 mg, the mean urinary recovery in the two 24 h collections preceding the study was 12.8 ±11.6 and 16.0 ±11.7 mg respectively or 7.4 ±5.4 and 11.0 ± 12.9 % of the total dose. A similar amount (12.4 ±7.4 mg or 7.9 ±6.3% of the dose) was recovered in the 24 h period following oral dosing on the first study day (Table 8.9). Following the intravenous infusion of 40 to 250 mg frusemide a mean of 11.0 ±7.1 mg was recovered in the urine or 14.1 ±14.0% of the total dose infused (Table 8.10). As expected, the urinary recovery of frusemide was dependent on the residual renal function, and was positively correlated with the creatinine clearance following both oral and intravenous administration ($r = 0.89$, $p < 0.001$ and $r = 0.87$, $p < 0.01$, $p < 0.01$ respectively).

The urinary recovery of frusemide was significantly less in the patients with renal failure than in the healthy volunteers in whom 34.7 ±8.9% and 55.2 ±4.8%

($p < 0.001$) of the oral and intravenous doses respectively were recovered in the urine in 24 h. Furthermore, the renal clearance of frusemide in the patients was only 11.8 $\pm$21.6 ml.min^{-1} and 11.2 $\pm$19.4 ml.min^{-1} respectively following oral and intravenous dosing and this was dramatically less than the corresponding values in the healthy volunteers (93.2 $\pm$33.6 ml.min^{-1} and 76.5 $\pm$18.0 ml.min^{-1}, $p < 0.001$). In keeping with this the renal clearance of frusemide in subject 10 was normal reflecting the patients relatively good creatinine clearance of 56 ml.min^{-1}.

The non-renal clearance of frusemide

The non-renal clearance of frusemide was 43.8 $\pm$12.2 ml.min^{-1} in the patients with chronic renal failure. This was significantly less than the corresponding values of 61.4 $\pm$11.8 and 61.9 $\pm$20.4 ml.min^{-1} observed in the volunteers and CAPD patients respectively ($p < 0.05$).

Table 8.10. The twenty four hour urinary recovery and renal clearance of frusemide in 10 conservatively managed patients with chronic renal failure given 80 to 500 mg frusemide orally and 40 to 250 mg by intravenous infusion and the calculated non renal clearance of the intravenous dose. "1" and "2" refer to the two twenty four hour collections of urine in the two days before the start of the study.

No.	Frusemide recovery 1		Frusemide recovery 2		Frusemide recovery		Renal clearance	Non-renal clearance
	(mg)	(%)	(mg)	(%)	(mg)	(%)	($ml.min^{-1}$)	($ml.min^{-1}$)
1	5.5	6.9	4.9	6.1	7.8	9.8	3.0	
1*					4.0	10.1	2.7	23.8
2	8.8	3.5	11.0	4.4	8.2	3.3	3.1	
2*					9.8	7.9	3.2	37.7
3	11.9	7.4	12.5	7.8	10.5	6.6	6.9	
3*					11.1	13.9	5.9	26.0
4	10.0	4.2	9.6	4.0	10.6	4.4	3.8	
4*					6.3	5.3	2.7	49.3
5	16.3	6.5	22.7	9.1	17.7	7.1	4.4	
5*					20.0	16.0	7.5	39.3
6	1.7	1.0	1.7	1.0	2.1	1.3	1.3	
6*					2.7	3.3	1.3	38.1
7	14.6	12.2	18.6	15.5	10.7	9.0	5.4	
7*					7.0	11.6	6.5	49.6
8	45.0	18.0	35.0	14.0	29.4	11.8	13.1	
8*					22.1	17.7	12.2	56.8
9	3.9	0.9	6.7	1.3	7.3	1.5	1.2	
9*					5.1	2.0	1.2	58.1
10	10.9	13.6	37.7	47.2	19.2	23.9	75.9	
10*					21.4	53.5	68.5	59.6
mean	12.8	7.4	16.0	11.0	12.4	7.9	11.8	
std	11.6	5.5	11.7	12.9	7.4	6.3	21.6	
mean*					11.0	14.1	11.2	43.8
std*					7.1	14.0	19.4	12.2

* = intravenous

SECTION 8.4: DISCUSSION

In patients with chronic renal failure taking once daily doses of frusemide 80 to 500 mg, all blood samples taken before the morning dose contained residual amounts of the drug from the doses of the previous day. In most studies in which the disposition of oral frusemide in patients with chronic renal failure has been investigated, single doses have been used in patients not already taking frusemide (Boutron et al., 1981, Huang et al., 1974 and Tilstone et al., 1978). Even when the patients were taking long term frusemide, the diuretic was usually stopped for up to a week before the start of the study (Kelly et al., 1977, Kühnel et al., 1987 and Rane et al., 1978). There is little information on the accumulation of frusemide during chronic oral dosing in patients with chronic renal failure, but the present findings are in agreement with those of Riva et al., (1982). In 7 children with end-stage renal failure on haemodialysis receiving 25 to 75 mg frusemide 2 or 3 times a day, the trough blood concentrations ranged from 0.71 to 1.94 $mg.l^{-1}$. Furthermore drug concentrations were similar at 0 and 8 h after dosing indicating that there was little or no accumulation of the drug. This presumably reflects compensatory non-renal clearance of frusemide as the contribution of haemodialysis to drug elimination was minimal.

Following the oral administration of frusemide in the present patients study, absorption took place rapidly with a measurable lag in only 3 of the 10 patients. Therefore, in most patients absorption had begun within 15 min and the mean T_{max} was 102 $\pm$46 min. The absorption process was virtually complete by 2 h and the bioavailability of oral frusemide was just over 60 % (61.3 $\pm$18.9 %).

The rapid absorption of frusemide in these patients was similar to that observed in the healthy volunteers. However, in the CAPD patients the lag time was significantly longer and absorption was not complete for 4 h. Despite this, the mean T_{max} was not

significantly longer and the mean bioavailabilty was similar in the CAPD patients.

The absorption of frusemide has frequently been noted to be delayed in patients with chronic renal failure as discussed in the previous chapter (Huang et al., 1975, Beerman et al., 1977, Boutron et al., 1981 and Riva et al., 1982). However in one of the few studies involving multiple doses of frusemide, the T_{max} was short at 49.8 $\pm$4.8, 86.4 $\pm$54.6 and 58.8 $\pm$27.6 min with 40, 80 and 250 mg frusemide respectively taken 4 times a day for 72 h. It is difficult to see how chronic administration of frusemide would lead to more rapid absorption, however, and both Riva et al., (1982) and Beerman et al., (1977) reported delayed absorption in patients on regular maintenance therapy. The differences in absorption observed in the conservatively managed and CAPD patients may simply reflect inter-subject variation and this has been invoked to explain individual differences in frusemide disposition in healthy volunteers (Boles Ponto and Schoenwald, 1990). In keeping with this is the long T_{max} observed in some of the conservatively managed patients. Alternatively it may reflect genuine differences in absorption in patients on CAPD.

Following the intravenous infusion of frusemide the plasma concentration-time data were best described by a two compartment model. The V_d was similar to that observed in the healthy volunteers and CAPD patients. With both oral and intravenous administration of frusemide, the elimination half life was significantly longer than in the healthy volunteers but were remarkably similar to those observed in the CAPD patients. As discussed in the previous chapter the present findings are in good agreement with the reported values in the literature of 2 to 4 h (Table 7.11). As expected, the total clearance of frusemide was significantly lower than in the healthy volunteers.

In most cases, there was very little frusemide in the urine following both oral and intravenous administration and the recovery was dependent on the severity of the renal

failure. The renal clearance was much lower than normal. This partly explains why much larger than normal doses may be needed in patients with renal failure in order to achieve sufficient concentrations in the lumen of the kidney to elicit a therapeutic response (Chennevasin et al., 1979).

The urinary recovery of frusemide in patients with chronic renal failure is usually very low. Beerman et al., (1977) administered 2 and 3 g frusemide respectively to 2 patients with end-stage renal failure and recovered only 1.4 and 0.7 % of the dose in the urine. Similarly, in a group of CAPD patients the urinary recovery of frusemide was 4.7 $\pm$1.1 and 5.8 $\pm$0.9 % of a 500 and 1000 mg dose respectively (Boutron et al., 1981).

Following the intravenous administration of frusemide 120 to 160 mg to patients with severe renal impairment (creatinine clearances 5 to 18 $ml.min^{-1}$), the mean urinary recovery was 11 $\pm$2 % of the dose (Brater et al., 1986, (b)). A similar percentage was recovered in patients given 40 or 80 mg intravenously whose plasma creatinine concentrations ranged from 250 to 1150 $\mu mol.l^{-1}$ (Traeger et al., 1984) and in patients given 80 to 600 mg with creatinine clearances of 3 to 27 $ml.min^{-1}$ (Voelker et al., 1987).

The renal clearance of frusemide in patients with impaired renal function has been described and is summarised in Chapter 7, Table 7.11. The present findings are in agreement with reported low values in patients with severe or end-stage renal failure which range from less than 1 to 9 $ml.min^{-1}$ (Beerman et al., 1977, Huang et al., 1974, Keller et al., 1981, Rane et al., 1978, Riva et al., 1982, Tilstone et al., 1978, Traeger et al., 1984 and Voelker et al., 1987).

The non-renal clearance of frusemide was significantly lower in the patients with

chronic renal failure than in the normal volunteers or CAPD patients. Similarly, Rane et al., (1978) reported a 41 % reduction in the non-renal clearance of frusemide in patients with uraemia compared to normal volunteers. However, in 3 other studies no differences were noted (Beerman et al., 1977, Keller et al., 1981 and Kelly et al., 1977). In contrast, Cutler et al., (1974) noted a marked increase in the non-renal clearance of frusemide in patients with chronic renal failure compared to normal volunteers and the non-renal clearance of frusemide has been shown to be increased in the nephrotic syndrome (Keller et al., 1982 and Smith et al., 1985).

The non-renal clearance of frusemide accounts for about 50 % of the total clearance of frusemide in normal circumstances (Chapter 6). Although non-renal clearance partly comprises glucuronide conjugation, other unknown mechanisms account for the rest (Branch, 1983). In healthy subjects about 14 % of an oral and intravenous dose of frusemide was excreted in the urine as the glucuronide conjugate and corresponding amount in kidney transplant patients was 8 % (Smith et al., 1980 (a) and Smith and Benet, 1983).

Although the non-renal clearance of frusemide was previously thought to occur predominantly by metabolism its disposition is not altered in cirrhosis (Fuller et al., 1981, Sawhney et al., 1981 and Verbeek et al., 1982). In the dog the non-renal clearance of frusemide was unaltered when the entire liver was removed and that the urinary recovery of frusemide glucuronide was unchanged (Verbeek et al., 1981 (b)). On the basis of these findings it has been suggested that glucuronidation may take place in extrahepatic sites including the kidney and that the liver does not contribute significantly to the non-renal component of its elimination (Branch 1983).

Probenecid pre-treatment in healthy subjects reduces both the renal and non-renal clearances of frusemide (Chennavasin et al., 1979) although in a later study a signifi-

cant effect was only shown on the renal clearance (Smith et al., 1980 (b)). Probenecid might decrease active transport of frusemide into the gut and that accumulated organic acids might have the same effect in chronic renal failure (Branch 1983). However, intestinal perfusion studies performed in healthy volunteers during intravenous frusemide administration showed that its clearance from the gastrointestinal tract was only 2 $ml.min^{-1}$ compared to a renal clearance of over 90 $ml.min^{-1}$ (Valentine et al., 1986). Finally it is possible that the other drugs that the patients were taking had an effect on the non-renal clearance or metabolism of frusemide.

In patients with conservatively managed chronic renal failure taking regular maintenance doses of frusemide, significant accumulation of the drug was not observed. The absorption and distribution of frusemide was similar to that observed in the healthy volunteers. Despite the large doses of frusemide administered to the patients, little appeared in the urine and recovery was dependent on residual renal function. The half life was prolonged and the plasma clearance was much lower than normal due not only to the expected reduction in the renal clearance but also to a smaller but significant reduction in the non-renal clearance.

CHAPTER 9.

THE INTERACTION OF PARACETAMOL WITH FRUSEMIDE

SECTION 9.1: INTRODUCTION

Frusemide produces a marked increase in the excretion of renal prostaglandins which may mediate at least some of its pharmacological actions such as the rise in plasma renin activity and natriuresis (Abe et al., 1977 and Scherer and Weber, 1979). In support of this the nonsteroidal anti-inflammatory drugs which inhibit prostaglandin synthesis have been shown to decrease the transient rise in plasma renin activity induced by frusemide (Patak et al., 1975, Rumpf et al., 1975, Fröhlich et al., 1976 and Passmore et al., 1989).

There is more controversy regarding the effects of the nonsteroidal anti-inflammatory drugs on the natriuretic response to frusemide (Atallah, 1979). Some investigators have reported a reduction when these drugs were coadministered (Patak et al., 1975, Fröhlich et al., 1976, Favre et al., 1983, Mackay et al., 1984 and Passmore et al., 1989) but others have found no such interaction (Williamson et al., 1975 (a), Bailie et al., 1976, Weber et al., 1977 and Riley et al., 1985).

Paracetamol has been considered in the past to be only a weak inhibitor of prostaglandin synthesis (Brune, 1983). However, the administration of paracetamol to healthy female volunteers under conditions of controlled sodium intake caused a reduction in PGE_2 excretion (Prescott et al., 1990) . This was associated with a reduction in sodium excretion and a delay in the onset of diuresis following an acute water load suggesting that paracetamol may share at least some of the properties of the other nonsteroidal anti-inflammatory drugs . This is particularly important in patients with impaired renal function who are often recommended to take paracetamol because it is considered to be a "safe" analgesic in such patients.

The purpose of this study was to investigate the effects of paracetamol pretreatment on

the renal excretion of prostaglandins following an intravenous dose of frusemide. The effect of paracetamol on frusemide-induced diuresis, natriuresis and rise in plasma renin activity was also studied to determine whether it had the potential to interfere with these actions in patients taking both drugs.

SECTION 9.2: METHODS

Volunteers

Ten healthy female volunteers of mean age 32 yr (range 22 - 47) and weight 72 kg (range 46 - 101) were studied. They had no medical illness and physical examination was normal. They were taking no regular medications other than the oral contraceptive pill, they did not smoke and they all claimed to drink less than 5 units of alcohol per week. The results of routine biochemical and haematological screening tests are presented in Tables 9.1 and 9.2 respectively.

Experimental design

Following the administration of either placebo or paracetamol for two days the effects of an intravenous dose of frusemide were studied on a third day after a final dose of either placebo or paracetamol. All subjects received both treatments which were given under single blind conditions in random order. Placebo (two lactose tablets) or 1 g paracetamol (two 0.5 g "Panadol" tablets) were taken at 08.30, 12.30, 16.30 and 20.30 h for two days. Food was avoided for 2 h before and for 2 h after dosing. To avoid problems with premenstrual fluid retention, the studies were carried out during the eighteen days following the end of a normal menstrual period. At least a week separated both studies.

The volunteers were instructed by the hospital dietician how to restrict their intake of sodium to 75 mmol per day for the two days before and during the period of drug

administration. Volunteers were asked to avoid sexual activity before the third study day on each occasion and to take no other drugs apart from the oral contraceptive pill for seven days before or during the study.

On the third day of the study on both occasions the fasting volunteers attended at the Clinical Pharmacology Unit at 08.00 h. An intravenous cannula was placed in a fore-arm vein and 200 ml of water was taken. The volunteers then remained recumbent for 30 min and 10 ml of venous blood was sampled for basal plasma renin activity (PRA activity). At 08.30 h the bladder was emptied and a final dose of either placebo or paracetamol 1 g was taken with 200 ml water. The patients remained recumbent and 1 h later (09.30 h) blood was again sampled for PRA activity and a urine sample collected.

Frusemide 20 mg (Hoechst U.K. Ltd.,) was then administered intravenously over 3 min via a butterfly needle in the opposite arm to the indwelling cannula. The volunteers drank 400 ml of water at this point and further amounts of 100 ml were then taken at 15 minute intervals for the next hour. Thereafter fluid was replaced according to the volume of urine passed at the 2 hourly collection points. Breakfast (toast and water or still orange juice) was served after the administration of frusemide and lunch was taken approximately 3 h later. No caffeine containing drinks were allowed during the day.

After the administration of frusemide blood was sampled at 15, 30 and 60 min for measurement of PRA activity. Urine was collected at 30, 60, 120, 240 and 360 min. Subjects were free to go at approximately 15.30 h and were asked to collect all urine passed until the end of the 24 h period after the administration of frusemide.

Table 9.1. Clinical biochemical test results in ten healthy female volunteers given 20 mg frusemide intravenously following treatment with either placebo or paracetamol (1 g four times per day) for two days.

	Plasma concentrations										
Subject No.	Protein $g.l^{-1}$	Albumin $g.l^{-1}$	Calcium $mmol.l^{-1}$	Phosphate $mmol.l^{-1}$	Alkaline phosphatase $u.l^{-1}$	Bilirubin $\mu mol.l^{-1}$	ALT $u.l^{-1}$	GGT $u.l^{-1}$	Bicarbonate $mmol.l^{-1}$	Creatinine $\mu mol.l^{-1}$	Urea $mmol.l^{-1}$
1	75	44	2.4	1.2	49	8	15	32	25	80	3.7
2	73	43	2.3	0.8	50	5	10	13	24	86	4.8
3	68	42	2.2	1.1	25	9	25	9	26	73	4.2
4	75	48	2.4	0.9	35	8	21	12	28	84	3.0
5	70	45	2.2	1.0	17	6	16	10	23	84	4.4
6	72	45	2.3	1.1	51	14	18	12	23	70	2.9
7	68	42	2.2	0.9	55	7	15	10	24	80	3.7
8	64	41	2.3	1.1	17	6	17	12	25	76	3.4
9	74	46	2.5	1.0	75	10	15	10	26	76	6.6
10	71	42	2.3	1.1	71	13	18	10	25	68	4.1
Normal range	60-80	36-47	2.1-2.6	0.8-1.4	40-100	2-17	10-40	10-55	24-30	55-150	2.5-6.6

Table 9.2. Results of haematological tests in 10 healthy female volunteers given 20 mg frusemide intravenously following treatment with either placebo or paracetamol (1 g four times per day) for two days.

Subject No.	Haemoglobin concentration $g.l^{-1}$	white cell count $\times 10^9.l^{-1}$	platelet count $\times 10^9.l^{-1}$
1	12.2	7.8	215
2	14.4	7.2	258
3	14.0	10.5	342
4	13.2	3.6	220
5	11.3	8.0	192
6	12.9	5.3	242
7	13.4	3.9	253
8	12.9	5.8	328
9	13.8	3.3	338
10	13.2	6.2	194
Normal range	13-18	4-11	150-350

Samples

All samples were processed as described in Chapter 2.

Assay of samples

Plasma renin activity was measured as described in Chapter 2. Urinary sodium was measured by an ion selective electrode, urinary frusemide by HPLC and urinary prostaglandins PGE_2 and 6-keto $PGF_{1\alpha}$ by radioimmunoassay (Chapter 2).

Statistics

Two way analysis of variance (ANOVA) was used to assess the significance of the effects of frusemide on each day. The significance of differences between the effects of frusemide with either placebo of paracetamol pre-treatment was assessed by split plot design ANOVA and 1-way ANOVA where appropriate.

SECTION 9.3: RESULTS

Frusemide induced diuresis

The mean urinary volumes following the administration of 20 mg frusemide intravenously are shown in Table 9.3 and Fig 9.1. In the hour following the administration of placebo or paracetamol the mean urinary volumes were 2.9 $\pm$2.1 $ml.min^{-1}$ (n=9) and 3.8 $\pm$3.1 $ml.min^{-1}$ (n=9) respectively. Subject number 1 failed to pass urine in this h. Frusemide administration caused a highly significant diuresis at 30 min of 23.9 $\pm$2.5 $ml.min^{-1}$, $p<0.001$, which was not altered by pre-treatment with paracetamol (23.0 $\pm$5.8 $ml.min^{-1}$). A significant diuresis of 18.6 $\pm$4.9 and 7.6 $\pm$3.3 $ml.min^{-1}$ was maintained at 60 and 120 min respectively which was similar with paracetamol pre-treatment (16.6 $\pm$5.1 and 7.6 $\pm$2.6 $ml.min^{-1}$). By 4 h after the dose of frusemide the urinary outputs had returned to basal values in both cases.

Frusemide induced natriuresis

The mean urinary sodium excretion rates following the administration of frusemide is shown in Table 9.3 and Fig 9.2. The mean basal excretion rates in the h after placebo or paracetamol were 232 $\pm$420 μmol.min^{-1} (n=8) and 241 $\pm$377 μmol.min^{-1} (n=9). Subject number one failed to pass urine at this point on either day and subject number 4 passed only 8 ml which was insufficient for analysis. The administration of frusemide induced a highly significant natriuresis at 30 and 60 min compared to the control sodium excretion rate (1998 $\pm$415 and 1336 $\pm$375 μmol.min^{-1}, $p<0.001$ respectively). With paracetamol pre-treatment the natriuresis was greater at 30 min (2192 $\pm$682 μmol.min^{-1}) and less at 60 min (1133 $\pm$356 μmol.min^{-1}) but the differences were not significant. The mean sodium excretion rate at 120 min was similar to the basal rate with either placebo or paracetamol pre-treatment (329 $\pm$127 and 339 $\pm$134 μmol. min^{-1}, respectively). However the next two urine collections showed an apparent rebound with reduced excretion rates of sodium at 6 h with placebo (61 $\pm$47 μmol.min^{-1}) or paracetamol pre-treatment (70 $\pm$24 μmol.min^{-1}).

Urinary excretion of prostaglandin E_2 (PG E_2) and 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$)

The urinary excretion rate of PGE_2 is shown in Table 9.3 and Fig 9.3. The concentrations of PGE_2 in subject number 2 were extremely high on the first study day and it was assumed that sexual intercourse had taken place. The data from this subject were therefore omitted for both days. The mean basal urinary excretion rate of PGE_2 was higher with placebo than with paracetamol pre-treatment (18.5 $\pm$14.3, n=7 compared with 7.6 $\pm$4.3 ng.h^{-1}, n=8, respectively) but the differences failed to reach statistical significance. Following the administration of frusemide the rate of excretion of PGE_2 increased in the first h to 34.5 $\pm$32.0 ng.h^{-1} with placebo and this effect was significantly blunted by paracetamol pre-treatment (16.4 $\pm$7.2 ng.h^{-1}, $p<0.05$).

Thereafter the excretion rates varied little between both study days. With placebo pre-treatment there was a rebound decrease in the output of PGE_2 at 2 h and although this had increased by 6 h it was still lower than the basal rate (13.1 $\pm$6.9 $ng.h^{-1}$). With paracetamol pre-treatment there was no rebound decrease in output of PGE_2 at 2 h and at this time the output was similar to basal levels at 9.3 $\pm$3.7 $ng.h^{-1}$.

The corresponding excretion rates of 6-keto $PGF_{1\alpha}$ are shown in Table 9.3 and Fig 9.4. The mean basal excretion rate of 6-keto $PGF_{1\alpha}$ was also higher with placebo than paracetamol (61.7 $\pm$38.5, n=8 compared with 38.7 $\pm$24.4 $ng.h^{-1}$ n=9, respectively) but the differences were not significant. In the 30 min following the administration of frusemide there was a transient increase in the urinary excretion rate of 6-keto $PGF_{1\alpha}$ which was less with paracetamol pretreatment (101.2 $\pm$63.7 v 64.2 $\pm$30.3 $ng.h^{-1}$). Again these differences were not statistically significant. At each subsequent collection point the mean excretion rate was always higher with placebo pre-treatment but not significantly. Nonetheless, the cumulative output over the first 2 h after frusemide was significantly lower with paracetamol than with placebo pre-treatment (137.1 $\pm$99.9 v 72.0 $\pm$33.8 $ng.h^{-1}$, $p<0.05$).

Urinary recovery of frusemide

The urinary recovery of frusemide following intravenous administration is given in Table 9.3 and Fig 9.5. In the first 30 min 5.9 $\pm$1.4 mg was recovered in the urine and with paracetamol the corresponding recovery was significantly higher at 7.2 $\pm$1.5 mg ($p<0.05$). Subsequently, the recovery of frusemide was much less and was not altered by paracetamol pre-treatment. The mean 24 h recovery was 11.6 $\pm$1.6 mg with placebo and 12.7 $\pm$1.4 mg with paracetamol ($p<0.05$).

Plasma renin activity

The mean PRA activity following the administration of frusemide is shown in Table 9.4 and Fig 9.6. When the volunteers had rested for 30 min the basal PRA activity was 2.0 $\pm$1.4 $ng.ml^{-1}.h^{-1}$ with placebo and 1.5 $\pm$0.6 $ng.ml^{-1}.h^{-1}$ with paracetamol pretreatment. One h later the values were very similar following the final dose of either placebo or paracetamol (1.7 $\pm$0.9 v 1.4 $\pm$0.5 $ng.ml^{-1}.h^{-1}$). With placebo, frusemide induced a significant increase in PRA activity at 15 (3.4 $\pm$2.7 $ng.ml^{-1}.h^{-1}$, $p<0.01$), 30 (3.5 $\pm$2.1 $ng.ml^{-1}.h^{-1}$, $p<0.001$) and 60 min (4.3 $\pm$2.9 $ng.ml^{-1}.h^{-1}$, $p<0.001$). The response was significantly reduced with paracetamol pre-treatment and the corresponding values at 30 and 60 min were 2.3 $\pm$1.4 and 2.7 $\pm$1.9 $ng.ml^{-1}.h^{-1}$ respectively, ($p<0.01$ compared with placebo).

Table 9.3. The diuresis, natriuresis, urinary excretion rate of prostaglandins PGE_2 and 6-keto $PGF_{1\alpha}$ and frusemide observed following the administration of 20 mg frusemide intravenously to ten healthy female volunteers pre-treated with either placebo or paracetamol* (1 g 4 times per day) for 2 days. Results are expressed as mean $\pm$s.d.

Time (min)	0	30	60	120	240	360	1440
Volume ml	2.9 ±2.1	23.9 ±2.5	18.6 ±4.9	7.6 ±3.3	2.7 ±1.6	2.6 ±1.4	1.0 ±0.5
*	3.8 ±3.1	23.0 ±5.8	16.6 ±5.1	7.6 ±2.6	3.0 ±1.2	3.7 ±1.2	0.9 ±0.5
Sodium $\mu mol.min^{-1}$	232 ±420	1998 ±415	1336 ±375	329 ±127	60 ±47	61 ±47	26 ±9
*	241 ±377	2192 ±682	1133 ±356	339 ±134	73 ±43	70 ±24	31 ±21
PGE_2 $ng.h^{-1}$	18.5 ±14.3	46.6 ±48.0	20.1 ±17.9	8.1 ±6.0	7.3 ±3.3	13.1 ±6.9	8.8 4.3
*	7.6 ±4.3	22.3 ±13.1	10.4 ±4.3	5.9 ±1.6	10.7 ±10.8	9.3 ±3.7	8.3 ±2.2
6-keto $PGF_{1\alpha}$ $ng.h^{-1}$	61.7 ±38.5	101.2 ±63.7	74.6 ±51.4	49.3 ±63.1	37.1 ±27.3	47.3 ±28.0	51.5 ±33.7
*	38.7 ±24.4	64.2 ±30.3	38.3 ±19.3	20.8 ±11.6	18.2 ±11.2	38.5 ±38.0	38.4 ±26.2
Frusemide mg	0.0 ±0.0	5.9 ±1.4	2.5 ±0.5	1.7 ±0.5	1.2 ±0.5	0.3 ±0.2	0.0 ±0.0
*	0.0 ±0.0	7.2 ±1.5	2.3 ±0.5	1.9 ±0.4	1.1 ±0.3	0.4 ±0.4	0.0 ±0.0

Table 9.4. The plasma renin activity ($ng.ml.h^{-1}$) observed in 10 healthy female volunteers given frusemide 20 mg intravenouly at time 0 following pretreatment with placebo and paracetamol (1 g 4 times per day) for 2 days with a final dose at time -60. Results are expressed as mean $\pm$s.d.

Time mins	-60	0	15	30	60
Placebo pretreatment	2.0 ±1.4	1.7 ±0.9	3.4 ±2.7	3.5 ±2.1	4.3 ±2.9
Paracetamol pretreatment	1.5 ±0.6	1.4 ±0.5	2.4 ±1.5	2.3 ±1.4	2.7 ±1.9

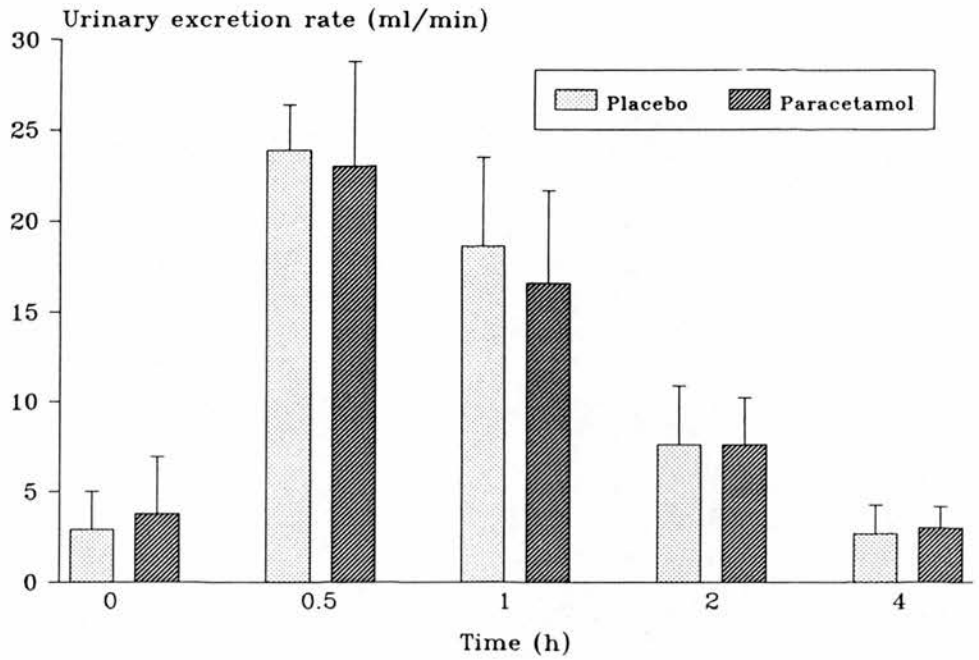


Fig 9.1. The diuresis (mean ±s.d.) induced by intravenous frusemide 20 mg in 10 volunteers pretreated with placebo or paracetamol (1 g 4 times a day) for 2 days.

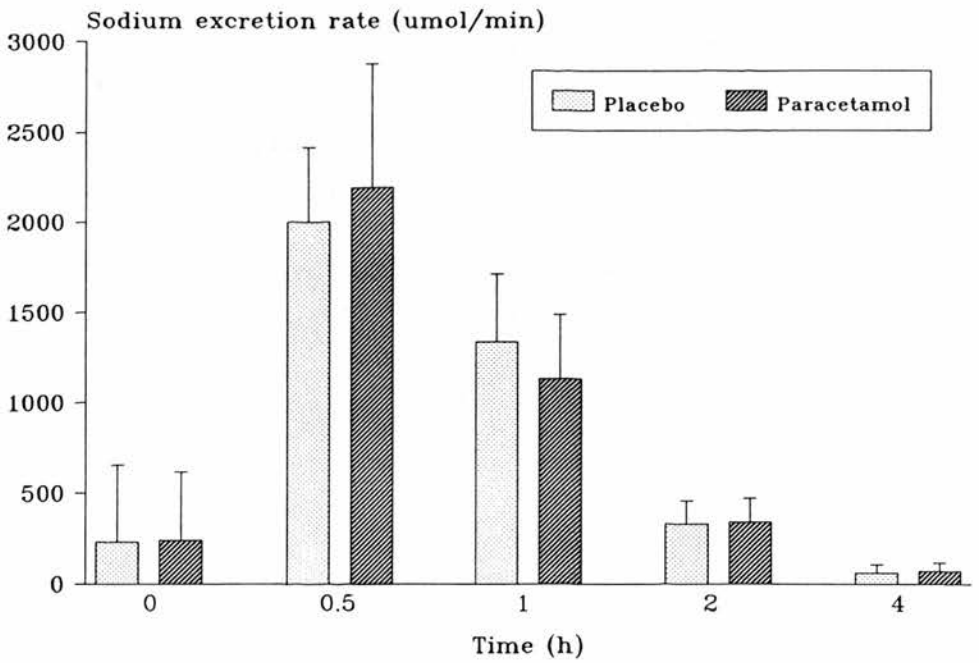


Fig 9.2. The natriuresis (mean ±s.d.) induced by intravenous frusemide 20 mg in 10 volunteers pretreated with placebo or paracetamol (1 g 4 times a day) for 2 days.

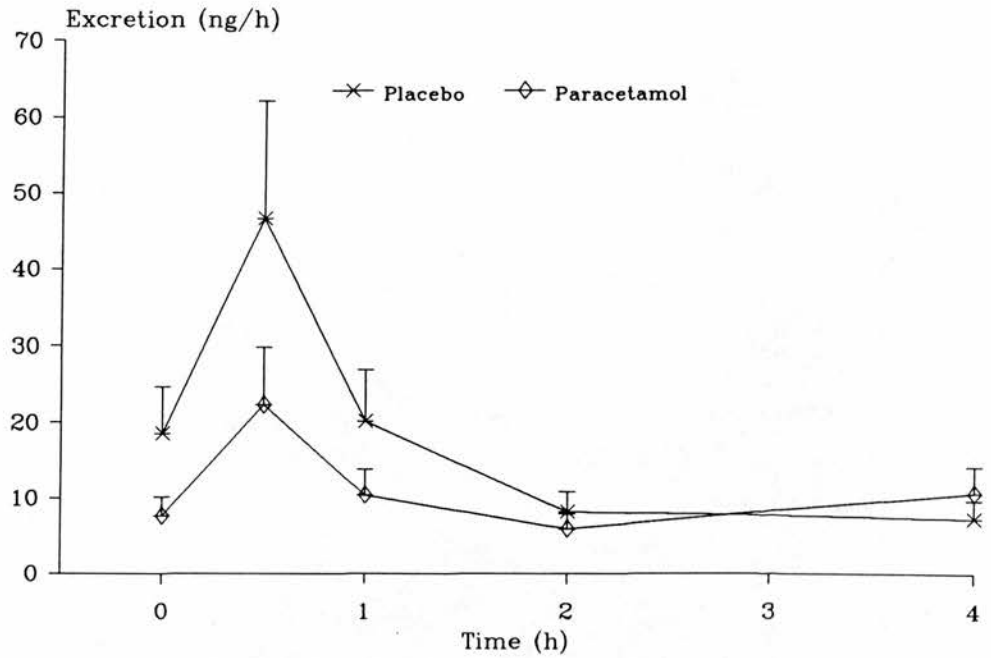


Fig 9.3. The urinary output of prostaglandin E_2 (mean ±s.e.m.) following 20 mg intravenous frusemide in 10 volunteers pretreated with placebo or paracetamol.

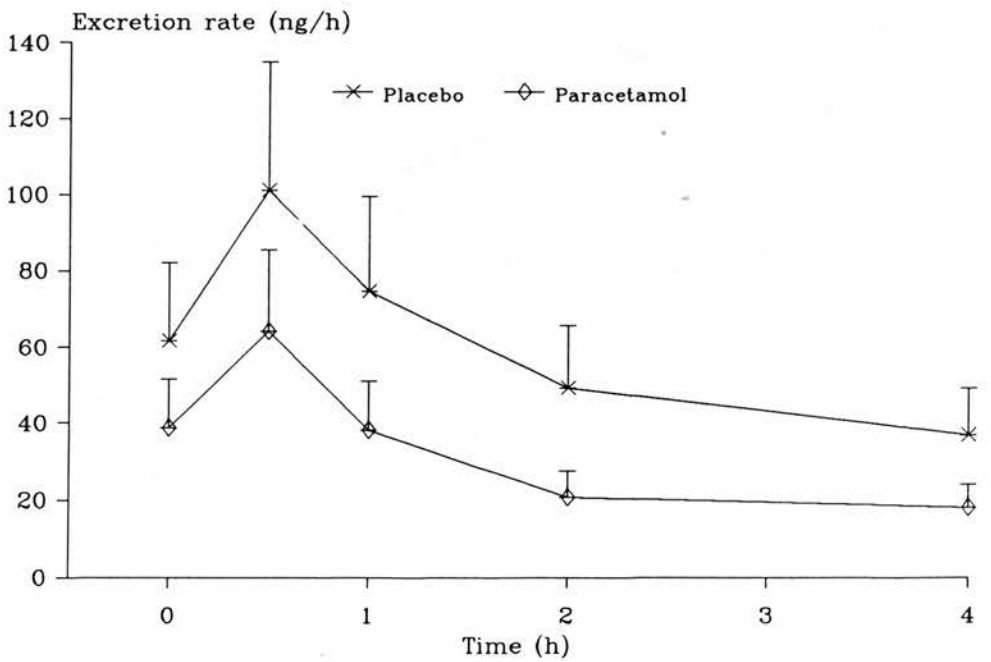


Fig 9.4. The urinary output of prostaglandin $F_{1\alpha}$ (mean ±s.e.m) following 20 mg intravenous frusemide to 9 volunteers pretreated with placebo or paracetamol.

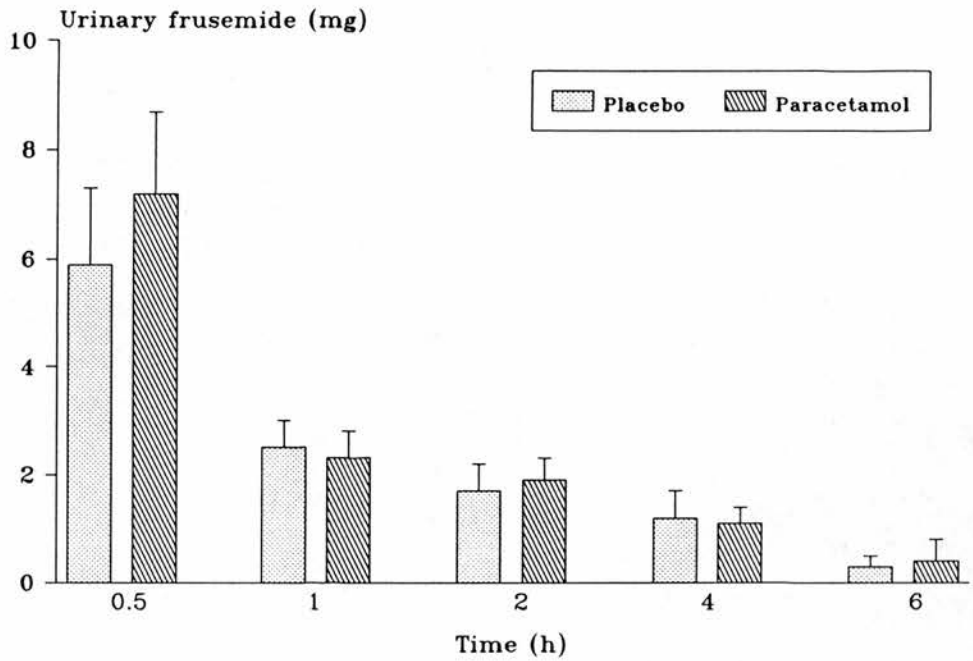


Fig 9.5. The urinary recovery of frusemide (mean ±s.d.) following an intravenous dose of 20 mg in 10 volunteers pretreated with placebo or paracetamol.

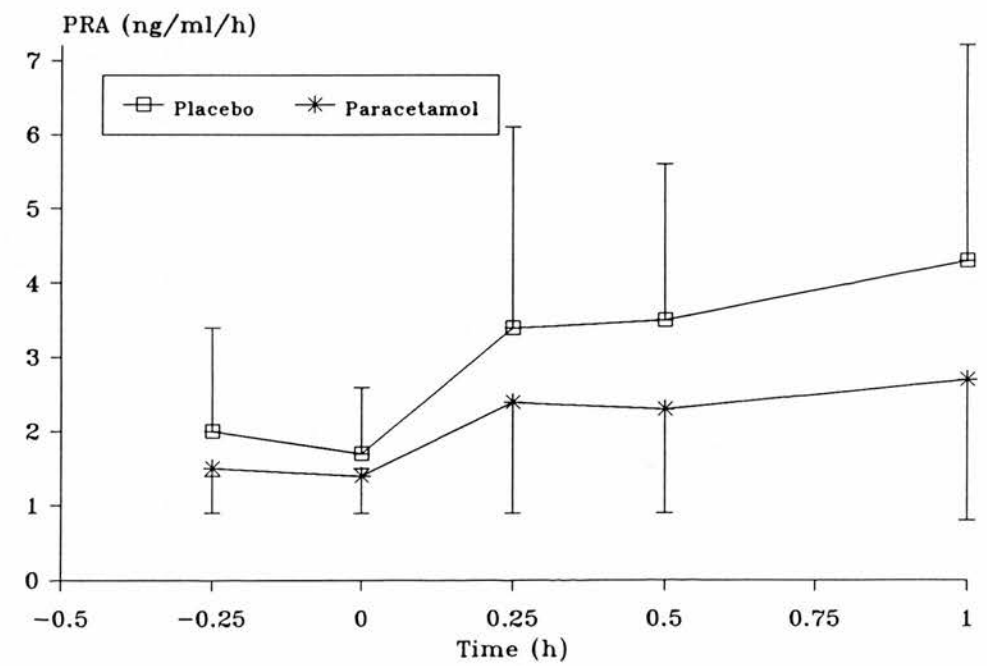


Fig 9.6. Plasma renin activity induced by 20 mg intravenous frusemide (mean ±s.d.) in 10 volunteers pretreated with placebo or paracetamol.

SECTION 9.4: DISCUSSION

The administration of 20 mg of frusemide intravenously to 10 healthy female volunteers caused a brisk natriuresis and diuresis which was not reduced by paracetamol pre-treatment. Frusemide also caused a transient rise in the urinary excretion of PGE_2 and 6-keto $PGF_{1\alpha}$ and a rise in plasma renin activity. These effects were significantly reduced by paracetamol.

At least some of the pharmacological actions of frusemide are mediated by the prostaglandins. Thus infusions of PGE_2 and prostacyclin (PGI_2) into the renal artery increase renal blood flow, sodium and potassium excretion and renin release (Fitzgerald et al., 1980, Gerber et al., 1978, Jackson et al., 1982, Jones et al., 1981, Martinez-Maldonado et al., 1972 and Schwertschlag et al., 1982). Williamson et al., (1975 (b)) first reported that the administration of frusemide to dogs was associated with increased levels of PGE_2 in renal venous blood and an increase in prostaglandin excretion following frusemide has also been shown in man (Abe et al., 1977, Scherer and Weber, 1979).

The loop diuretics might inhibit the metabolism of prostaglandins and this effect could be responsible for the prostaglandin-mediated effects of frusemide. Frusemide inhibits the enzyme PGE_2-9-ketoreductase which converts PGE_2 to the inactive $PGF_{2\alpha}$ and 15-hydroxy prostaglandin dehydrogenase which converts active prostaglandins to their inactive 15-keto metabolites (Stone and Hart, 1976, Paulsrud and Millar, 1974). However, as pointed out by Gerber (1983) these experiments were performed in cell-free systems derived from placenta and kidney and the concentration of frusemide necessary to produce 50% inhibition of the enzymes was 10 to 100 times higher than those achieved in humans. Furthermore, in man, frusemide increases the urinary excretion of all the prostaglandins and the increase in PGE_2 output is not at the ex-

pense of $PGF_{2\alpha}$ as would be expected with 9-ketoreductase inhibition (Ciabattoni et al., 1979). It seems more likely that frusemide causes an increase in de-novo synthesis of prostaglandins possibly due to the increased availability of the prostaglandin substrate arachadonic acid (Weber et al., 1977).

The rise in PRA activity activity induced by frusemide is prostaglandin-mediated (Gerber et al., 1981) and this effect is consistently blocked by the nonsteroidal anti-inflammatory drugs which inhibit the formation of prostaglandins (Patak et al., 1975, Rumpf et al., 1975, Fröhlich et al., 1976, Tan and Mulrow, 1977, Mackay et al., 1984, Riley et al.,1985 and Passmore et al., 1989). The relationship between the prostaglandin system and the natriuretic effect of frusemide is less clear-cut and there is considerable controversy regarding the effects of the nonsteroidal anti-inflammatory drugs on this response (Attallah, 1979). Some investigators have reported a reduced natriuretic response to frusemide when nonsteroidal anti-inflammatory drugs were given at the same time (Patak et al., 1975, Fröhlich et al., 1976, Favre et al., 1983, Passmore et al., 1989 and Mackay et al., 1984). However other reports showed no such interaction (Williamson et al., 1975 (a), Bailie et al., 1976, Weber et al., 1977 and Riley et al., 1985).

The natriuretic effect of the prostaglandins may be mediated by an increase in renal blood flow with redistribution towards medullary nephrons (Kirschenbaum et al., 1974). Alternatively, it has been proposed that PGE_2 exerts a direct effect on the collecting tubule or medullary thick ascending Loop of Henle (Stokes and Kokko, 1977 and Stokes, 1979). These effects however were only present when PGE_2 was added to the peritubular as opposed to the luminal surface of the nephron where frusemide exerts its action (Burg et al., 1973). Fine and Kirschenbaum (1981) were unable to reproduce these findings.

It is perhaps not surprising that several investigators failed to show a blunting of the natriuretic effect of frusemide with the nonsteroidal anti-inflammatory drugs. Differences between studies may be due to variations in protocol particularly with respect to fluid and sodium balance, and residual renal function (Riley et al., 1985). Sodium balance seems to be particularly important. In dehydrated dogs frusemide caused an increase in renal blood flow which was abolished by indomethacin. However in dogs treated with a high salt diet and deoxycorticosterone acetate (DOCA), a regime which inhibits the renal vascular effects of frusemide (Gerber, 1980), the administration of frusemide failed to cause vasodilitation and indomethacin did not affect renal blood flow (Nies et al., 1982). On the basis of these findings it has been suggested that the frusemide-induced diuresis and natriuresis that occurs in volunteers taking a normal sodium diet may not be mediated by prostaglandins (Riley et al., 1985). However, in most of the studies where there was a demonstrable inhibition of frusemide-induced natriuresis by the nonsteroidal anti-inflammatory drugs, the volunteers were taking either a diet containing 120 to 150 mmol sodium per day (Patak et al., 1975, Fröhlich et al., 1976, Favre et al., 1983 and Passmore et al., 1989) or an unrestricted sodium diet (Mackay et al., 1984). The role of the prostaglandins in the tubular response to frusemide is therefore tenuous and is most likely to be attributable to prostaglandin-mediated renal vasodilatation .

Paracetamol has been considered to be only a weak inhibitor of prostaglandin biosynthesis although it has been suggested that it may be more effective against enzymes in the central nervous system than those in the periphery (Flower and Vane, 1972). For this reason it is generally thought not to share the properties of the other nonsteroidal anti-inflammatory drugs such as fluid retention and interference with loop diuretics. However, the administration of paracetamol to healthy female volunteers under conditions of controlled sodium intake caused a reduction in PGE_2 excretion (Prescott et al., 1990). This was associated with a reduction in sodium output and a delay in the

onset of a diuresis following an acute water load. An earlier study by Haylor (1980) found that paracetamol caused a reduction in sodium and water output and it also has an antinatriuretic effect in patients with diabetes insipidus (Nusynowitz and Forsham, 1966). In contrast, Berg et al., (1990) found a reduction in sodium and PGE_2 output with paracetamol therapy in healthy elderly volunteers and not in young subjects.

These reports suggest that paracetamol may share at least some of the properties of the other nonsteroidal anti-inflammatory drugs. In the present study it was found that although paracetamol reduced the frusemide-induced increase in PGE_2 and 6-keto $PGF_{1\alpha}$, it had no effect on the natriuresis or diuresis. Paracetamol also reduced the PRA activity activity which is presumably related to its inhibition of renal prostaglandin synthesis. In the present study, significantly more frusemide was recovered in the urine when volunteers had taken paracetamol. However, this was not associated with a significant increase in the natriuresis or diuresis despite the fact that the action of frusemide is dependent on the amount present at the luminal side of the ascending loop (Burg et al., 1973). It is possible that paracetamol competitively inhibited glucuronide metabolism of frusemide so that relatively more unchanged frusemide was excreted in the urine.

The results of this study are of relevance to patients with impaired renal function. Paracetamol is widely used in patients with renal failure because it is considered to be a "safe" analgesic. However we have clearly shown that it has an effect on renal prostaglandins and in particular that it blunts the rise in the excretion of prostaglandins and in PRA activity induced by frusemide. Most, if not all, of the nonsteroidal anti-inflammatory drugs can cause sodium retention, development or aggravation of oedema, hyperkalaemia, interference with the actions of diuretics and ß-blockers and the development of renal failure, particularly under conditions of reduced renal perfusion (Garella and Matarese, 1984). There is evidence that paracetamol may be impli-

cated in the development of acute renal failure following overdose without severe liver damage (Kleinman et al., 1980, Coben et al., 1982 and Bjorck et al., 1988) and long term daily use is associated with an increase risk of chronic renal disease (Sandler et al., 1989). Paracetamol may therefore not be as safe an analgesic as was first thought and its use in patients with renal impairment, particularly those requiring diuretics, should be reviewed.

FINAL DISCUSSION

In the present study a number of changes in the disposition of the commonly used analgesic, paracetamol, and the loop diuretic, frusemide, were demonstrated in patients with chronic renal failure. Although some of the findings were predictable, others were not and could not be explained simply by the presence of renal impairment. It does appear, however, that patients with renal failure who are maintained on long term medication in high doses may absorb, metabolise and eliminate these drugs in unexpected ways. This may account for some of the variability in drug action and increased incidence of side effects often noted in such patients.

In patients with end-stage renal failure maintained on CAPD, the absorption of frusemide, but not paracetamol, was delayed. Despite this, the bioavailability of frusemide was not reduced and indeed it tended to be higher than in normal volunteers. On the other hand, conservatively managed patients with chronic renal impairment absorbed frusemide normally, and there was no evidence of dose-dependent absorption. It may be that absorption of frusemide is impaired only in the presence of severe renal failure because of more marked alterations in gastrointestinal motility or the presence of gut oedema. On the other hand absorption may be influenced by the presence of large volumes of dialysate in the peritoneal cavity. If this is the case it is difficult to account for the apparently normal absorption of paracetamol under similar circumstances and it may simply be a further manifestation of the previously noted marked inter-individual variation in the oral absorption and disposition of frusemide.

The peritoneal membrane is an effective method of removing uraemic waste products but contributes little to the clearance of most drugs as demonstrated for paracetamol and frusemide. The clearance by peritoneal dialysis even of those drugs or drug metabolites which have a low volume of distribution and little binding to plasma proteins will be very small and limited by the "low flow" rate of the peritoneal effluent. This was certainly the case for the polar glucuronide and sulphate metabolites of

paracetamol which are normally readily excreted by the kidney. The peritoneal membrane could therefore not effectively eliminate these conjugates during chronic dosing in patients with end-stage renal failure and marked accumulation would seem inevitable under such circumstances. The clearance of frusemide by the peritoneal membrane was minimal, possibly because of high plasma protein binding. Marked accumulation of frusemide would not occur during chronic dosing in CAPD patients, however, because of substantial non-renal clearance.

The metabolism of both paracetamol and frusemide may have been affected in patients with chronic renal failure. Thus, in conservatively managed patients taking regular doses of paracetamol over 10 days, the glucuronide but not the sulphate conjugate accumulated as predicted. This may simply have been a result of sulphate depletion caused by frequent administration of therapeutic doses of paracetamol, although this is less likely in patients with renal failure who tend to retain sulphate. There was also evidence of enterohepatic circulation of retained conjugates with some regeneration of paracetamol by hydrolysis in the gut. Although this is probably not of great importance with paracetamol, similar regeneration of the parent compound with other drugs could be of vital clinical significance.

In patients with end-stage renal failure maintained on haemodialysis taking a similar regime of paracetamol neither the glucuronide nor the sulphate conjugate accumulated as predicted. It is difficult to explain these findings but the possibilities include poor compliance, reduced absorption of paracetamol, altered metabolism, increased biliary excretion of metabolites or greater than expected clearance of paracetamol during haemodialysis. It is unlikely that all of the patients would show poor compliance and the absorption of single doses of paracetamol in end-stage renal failure was normal. Altered metabolism is possible, particularly of the sulphate conjugate. Increased biliary excretion of the retained conjugates may also have occurred under such circum-

stances possibly with some regeneration of the parent compound by hydrolysis of the conjugates in the gut. The clearance of paracetamol glucuronide during haemodialysis was restricted to less than 40 $ml.min^{-1}$ and this certainly does not help to explain the findings. The most likely explanation is a compensatory increase in enterohepatic circulation of the polar conjugates of paracetamol during chronic dosing in these patients, but altered metabolism of paracetamol, perhaps to compounds which were not detected by the assay, is also possible.

There was also evidence that the metabolism of frusemide was altered in conservatively managed patients with chronic renal failure in that the non-renal clearance was reduced during chronic dosing. It was, however, unaltered in the CAPD patients following single doses. Although frusemide is partly metabolised to a glucuronide conjugate, most of the non-renal clearance occurs by unknown mechanisms which are not thought to involve the liver. Elimination of frusemide by this mechanism undoubtedly explains the lack of accumulation of frusemide observed in the conservatively managed patients despite the large daily doses of diuretic they required. It is possible that saturation of this pathway would occur during chronic dosing leading to the observed reduction in the non-renal clearance in the conservatively managed but not the CAPD patients who were not taking frusemide regularly. Indeed one of the striking findings was how little of the frusemide taken by the conservatively managed patients actually reached the active site at the tubular lumen.

The patients were all taking a variety of other medication and the observed changes in the disposition of paracetamol and frusemide could have been due to interactions with some of these. One of the expected findings was that paracetamol and frusemide themselves could interact with one another. Pretreatment with paractamol reduced the rise in plasma renin activity and increased excretion of urinary prostaglandins induced by intravenous frusemide but not the natriuresis or diuresis.

The present findings have important implications for drug prescribing in patients with chronic renal failure. Absorption of many drugs, including diuretics may be impaired. Furthermore, under conditions of chronic dosing metabolism may be abnormal with increased compensatory elimination of drug metabolites in the bile. The possibility then exists for regeneration of the parent compound by hydrolysis of conjugates in the gut with subsequent reabsorption resulting in higher than normal therapeutic, or toxic, plasma concentrations. Accumulated drug metabolites may also lead to unexplained toxicity in the presence of renal failure. The peritoneal membrane may not be relied upon to effectively eliminate drugs in CAPD patients as the clearance of drugs by this route is very limited. Haemodialysis may not eliminate polar drug metabolites as effectively as was thought and the reasons for this are unclear. Drug interactions may not always be obvious and the use of paracetamol in patients with chronic renal failure, particularly those requiring diuretics, should be reviewed.

Several important questions have arisen from these studies and much work still needs to be done. In particular, detailed absorption studies should be done in patients with chronic renal failure following both single and multiple doses of paracetamol and frusemide. A crucial area of research is to explain why paracetamol metabolites did not accumulate as predicted during chronic dosing and, in particular, to determine whether increased biliary excretion of retained metabolites was responsible. Such studies might involve the use of radiolabelled drug. In addition, measurement of plasma inorganic sulphate levels during multiple dosing might help to determine whether saturation of sulphate conjugation occurs in patients with renal failure under such circumstances. Elucidation of the mechanisms of the non-renal clearance of frusemide are required both in normal volunteers and renal failure patients and much work needs to be done to explain the marked differences in its disposition observed between individuals.

CONCLUSION

Paracetamol and frusemide provide very useful models of altered drug disposition in patients with renal impairment and the present studies underline our ignorance about how patients deal with the large doses and chronic administration of drugs required for therapeutic purposes. Many of the observed changes in disposition could be explained simply by the reduced renal function. However, some of the alterations in absorption, metabolism, metabolite accumulation and non-renal clearance including clearance by dialysis were completely unexpected and not readily explained. In addition, the studies illustrate how frequently commonly used drugs may interact with one another in unsuspected ways. Clearly great care is needed to ensure safe and appropriate drug prescribing in patients with chronic renal failure.

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ABBREVIATIONS

The following abbreviations have been used:

AUC	area under the plasma concentration-time curve
C	centigrade
C_t	drug plasma concentration at time "t"
C_{max}	the maximum concentration of drug in the body
g	gram
h	hour
K	the elimination rate constant of a drug
k_a	the absorption rate constant
k_d	the distribution rate constant
kg	kilogram
l	litre
M	molar
min	minute
mg	milligram
ml	millilitre
ng	nanogram
s.d.	standard deviation
s.e.m.	standard error of the mean
T_{max}	the time to reach peak plasma concentrations
$t_{½}$	the drug elimination half life
μl	microlitre
μmol	micromole
V_d	volume of distribution
yr	year

PUBLICATIONS RELATING TO THIS THESIS

ABSTRACTS FROM PRESENTATIONS TO SCIENTIFIC MEETINGS

Frusemide disposition in patients on continuous ambulatory peritoneal dialysis (CAPD).

U. Martin, R.J. Winney, L.F. Prescott.

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Cumulation of paracetamol and its metabolites during multiple dosing in patients with chronic renal failure.

U. Martin, R.M. Temple, R.J. Winney, L.F. Prescott.

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Interaction of paracetamol with frusemide.

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European Journal of Clinical Pharmacology

The disposition of paracetamol and the accumulation of its glucuronide and sulphate conjugates during multiple dosing in patients with chronic renal failure

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Summary. We have compared the disposition of oral paracetamol (1.0 g t.d.s. for 10 days) in 6 healthy volunteers and 6 conservatively-managed patients with chronic renal failure (mean plasma creatinine 451 $\mu mol \cdot l^{-1}$). Blood was sampled daily for 10 days before the morning dose of paracetamol.

Each day the pretreatment plasma concentrations of paracetamol were higher in the renal failure patients than in the volunteers, with mean values over the 10 days of 3.1 and 1.1 $mg \cdot l^{-1}$ respectively. The mean daily plasma concentrations of the sulphate and glucuronide conjugates of paracetamol were markedly higher in the renal failure group and apparent steady-state concentrations of about 25 and 85 $mg \cdot l^{-1}$ were reached on the 2nd and 6th days respectively. The mean steady-state plasma concentrations of the glucuronide conjugate on the 7th to 10th days of treatment were positively correlated with the plasma creatinine concentration ($r = 0.97$), but this relationship did not hold for the sulphate conjugate. Cysteine and mercapturate conjugates could only be detected in one patient.

Predictions of steady-state concentrations based on previous studies with single doses of paracetamol in renal failure patients were remarkably accurate for the glucuronide but not for the sulphate conjugate.

These results are consistent with some extra-renal elimination of retained paracetamol conjugates in patients with chronic renal failure, with limited regeneration of the parent compound. The sulphate metabolite did not accumulate as predicted, possibly because of depletion of inorganic sulphate.

Key words: Paracetamol, Renal failure, glucuronide conjugation, sulphate conjugation, multiple dosing, accumulation

The excretion of polar drug metabolites is impaired in patients with chronic renal failure, and when single doses of paracetamol were given to such patients the plasma concentrations of its glucuronide and sulphate conjugates were greatly increased compared with those observed in healthy volunteers [Prescott et al. 1989]. Furthermore, in anephric patients haemodialysis appeared to be the major route of elimination of paracetamol metabolites [Øie et al. 1975]. Therefore, when patients with moderate to severe renal impairment take paracetamol regularly over a long period of time, significant accumulation of its polar metabolites would be expected. Under these conditions it is possible that the retained conjugates might undergo enterohepatic circulation, with some regeneration of the parent compound by hydrolysis in the gastrointestinal tract [Verbeeck et al. 1981].

Little attention has been paid to the accumulation of drugs and their metabolites during long-term treatment of patients with impaired renal function. We have therefore compared the disposition of paracetamol and its metabolites in healthy volunteers and in conservatively managed patients with chronic renal failure taking 1 g of paracetamol three times a day for 10 days. The plasma concentrations of the glucuronide and sulphate conjugates were compared with those expected on the basis of previous single-dose studies [Prescott et al. 1989].

Materials and methods

Patients and volunteers

Studies were carried out in 6 healthy volunteers (3 men and 3 women), mean age 32 y (range 18–37 y) and mean weight 60 kg (range 48–75 kg). They had no significant past medical history and were not taking any regular medications. They did not smoke and denied heavy consumption of alcohol. Physical examination and routine biochemical and haematological screening were normal. We also studied 6 patients (5 men, 1 women) on conservative management for chronic renal failure. Their clinical details are summarized in Table 1. Their mean age and weight were 55 y (range 43–75 y) and 74 kg (range 52–97 kg). Apart from mild elevation of the alkaline phosphatase in two of the patients, liver function tests were normal. Both the patients and the volunteers were asked to avoid taking

Table 1. Clinical details of the patients with chronic renal failure

Patient	Age & sex	Weight (kg)	Medical & renal diagnoses	Haemoglobin ($g \cdot dl^{-1}$)	Albumin ($g \cdot l^{-1}$)	Plasma creatinine ($\mu mol \cdot l^{-1}$)	Creatinine clearance ($ml \cdot min^{-1}$)	Drugs
1	65M	84	Diabetes mellitus Diabetic nephropathy	10.2	39	590	18	insulin enalapril indapamide sodium bicarbonate
2	59M	97	Hypertension Glomerulonephritis	12.6	35	407	22	frusemide enalapril aspirin sodium bicarbonate
3	40M	67	Diabetes mellitus Diabetic nephropathy	13.7	40	312	42	insulin frusemide captopril metoprolol
4	50M	70	Hypertension Glomerulonephritis	11.7	33	379	18	nifedipine metoprolol
5	75M	76	Ileal loop diversion Pyelonephritis	12.2	42	247	29	isosorbide mono-nitrate sodium bicarbonate
6	43F	52	Pyelonephritis	10.7	41	774	8	alphacalcidol sodium bicarbonate, aluminium hydroxide

paracetamol for 7 days before and for 4 days after the study. The study was approved by the local Ethics Committee of Medical Research and all the subjects gave their informed consent before taking part.

Drug administration and blood sampling

The patients and volunteers took paracetamol 1 g (2 × 500 mg soluble Panadol® tablets) dissolved in approximately 100 ml of water, at 10.00, 16.00, and 22.00 h for 10 days. Food was avoided for 2 h before and for 2 h after dosing. Venous blood was sampled at 09.00 h on the first day of the study before starting paracetamol, at the same time each morning for the next 10 days, and on the mornings of the 2nd and 4th days after the last dose of paracetamol. The plasma was stored at −20°C until assayed for paracetamol and its glucuronide, sulphate, cysteine, and mercapturate conjugates by HPLC with UV and electrochemical detection [Clements et al. 1984].

Data analysis

Steady-state concentrations of paracetamol glucuronide and sulphate conjugates were predicted from the relationship between the degree of renal failure and the minimum steady-state concentrations calculated from data obtained in a previous single-dose study [Prescott et al. 1989]. The data were fitted to a single exponential by the "SIPHAR" pharmacokinetic program, taking the "dose" as the amount of conjugate formed from the administered paracetamol. Using the derived coefficients and exponents the minimum steady-state conjugate concentrations with the present repeated dose schedule were simulated according to the superimposition principle [Wagner 1975]. These concentrations of glucuronide and sulphate conjugates were closely related to the plasma creatinine concentration ($r = 0.89$ and 0.95 respectively, $P < 0.01$), and the corresponding regression equations were used to predict the minimum concentrations at steady-state in the present study.

Statistical differences between the groups were determined by the Mann-Whitney test and the level of significance was taken as $P < 0.05$. Correlations were determined using standard regression analysis. The results are given as means (SD) and the concentrations of the conjugates have been expressed as paracetamol equivalents.

Results

Paracetamol

The mean daily pretreatment plasma concentrations of paracetamol and its glucuronide and sulphate conjugates are shown in Table 2. Each day the plasma concentrations of paracetamol were higher in the renal failure patients than in the volunteers. The mean values over the 10 days of treatment of 3.1 (0.6) and 1.1 (0.3) $mg \cdot l^{-1}$ respectively ($P < 0.01$).

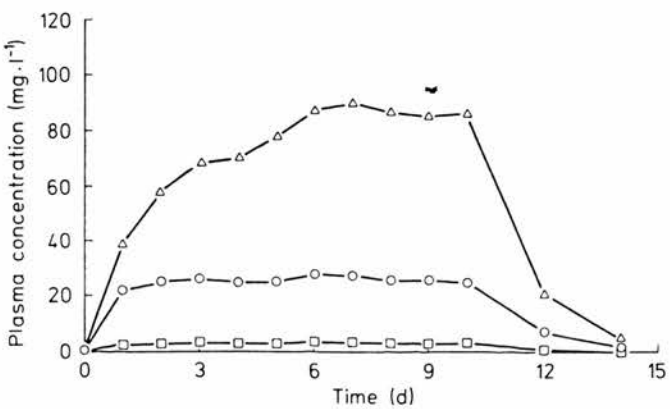


Fig. 1. Mean daily plasma concentrations of paracetamol (□) and its glucuronide (△) and sulphate (○) conjugates in 6 patients with chronic renal failure taking paracetamol (1 g t.d.s.) for 10 days □—□ Paracetamol △—△ Glucuronide ○—○ Sulphate

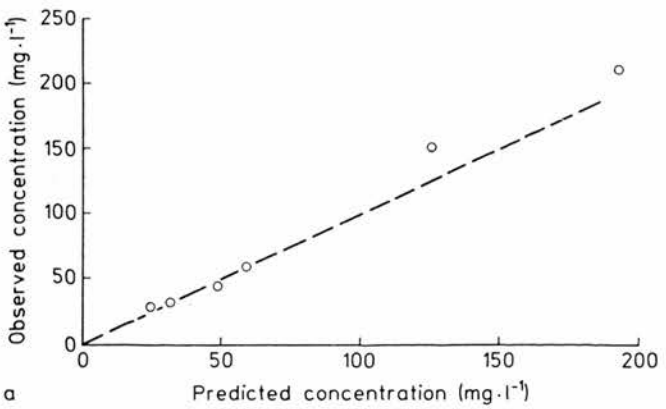
Table 2. Mean daily pre-dose plasma concentrations of paracetamol and of its glucuronide and sulphate metabolites ($mg \cdot l^{-1}$) in 6 healthy volunteers and 6 patients with chronic renal failure treated with paracetamol (1.0 g t.d.s.) for 10 days

	Day							
	1	2	3	4	5	6	7–10[a]	14
Paracetamol concentrations ($mg \cdot l^{-1}$)								
Healthy volunteers	1.3 (0.5)	1.4 (0.5)	1.2 (0.5)	1.2 (0.7)	1.7 (1.6)	1.5 (0.7)	0.8 (0.5)	ND
Renal failure patients	2.4 (0.4)	2.7 (0.7)	3.1 (1.1)	2.9 (0.9)	2.7 (1.5)	4.7 (3.4)	3.1 (1.3)	ND
Glucuronide concentrations ($mg \cdot l^{-1}$)								
Healthy volunteers	2.9 (0.8)	3.8 (1.3)	3.7 (0.9)	3.9 (1.4)	2.6 (1.5)	4.5 (1.3)	3.0 (0.5)	0.3 (0.6)
Renal failure patients	39 (19)	58 (36)	68 (47)	69 (51)	78 (61)	87 (67)	87 (69)	4.4 (7.0)
Sulphate concentrations ($mg \cdot l^{-1}$)								
Healthy volunteers	1.7 (0.6)	1.6 (0.4)	1.4 (0.6)	1.4 (0.6)	1.7 (1.0)	1.9 (0.9)	1.1 (0.4)	0.5 (0.3)
Renal failure patients	22 (11)	25 (16)	26 (19)	24 (17)	25 (21)	27 (28)	25 (19)	1.4 (1.9)

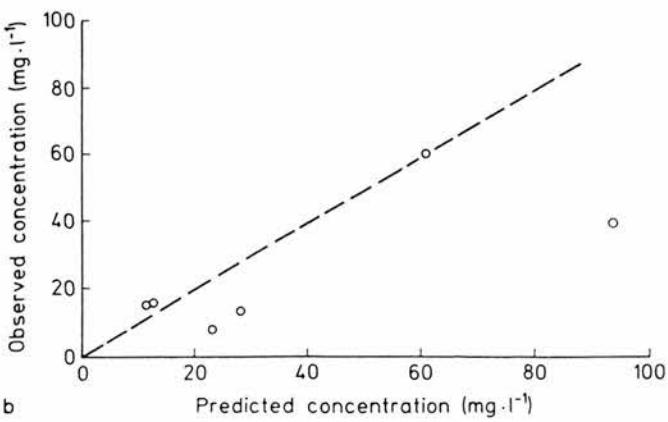
ND = not detectable
[a] mean of concentrations on days 7–10

Glucuronide and sulphate conjugates

As expected the mean daily concentrations of the conjugates were markedly higher in the patients than in the volunteers, and mean values from the 7th to the 10th day were over twenty times higher in the patients (Table 2).



a



b

Fig. 2 a, b. Steady-state plasma concentrations of (**a**) the glucuronide and (**b**) the sulphate conjugate of paracetamol measured in patients with chronic renal failure taking paracetamol (1 g t.d.s.) for 10 days plotted against steady-state concentrations of the (**A**) glucuronide and (**B**) the sulphate conjugate predicted from a previous single-dose study

The mean plasma concentration of the sulphate conjugate in the renal failure patients was 21.7 (11.2) mg^{-1} after the first 24 h and 24.5 (18.4) $mg \cdot l^{-1}$ by the 10th day of treatment with little daily variation (Fig. 1). In contrast, the mean plasma glucuronide concentrations increased progressively to 87 (67) $mg \cdot l^{-1}$ on day 6, after which there was no further accumulation. Thus, mean steady-state concentrations were reached for the sulphate and glucuronide conjugates by the 2nd and 6th days respectively. However, there was considerable interindividual variation in the concentrations of retained conjugates at steady state and in the time taken to reach it. The means of the concentrations measured on the 7th to 10th days were therefore taken to represent steady-state values. The steady-state concentration of the glucuronide conjugate were positively correlated with the plasma creatinine concentration ($r = 0.97$, $P < 0.01$, $n = 6$). However, the corresponding correlation between the steady-state concentrations of the sulphate conjugate and the plasma creatinine concentration was not statistically significant ($r = 0.74$, $P > 0.05$, $n = 6$).

Predicted glucuronide and sulphate conjugate concentrations

The minimum steady-state plasma concentrations of the glucuronide conjugate predicted from the previous single-dose studies were remarkably similar to the concentrations observed at steady state in the patients with renal failure (Fig. 2 a). However, predictions of the steady-state concentrations of the sulphate conjugate were accurate in only 3 of the 6 patients, and were less than 50% of those expected in the others (Fig. 2 b). In the patients in whom the predicted and observed concentrations of sulphate conjugates were similar the ratios of glucuronide to sulphate conjugates were less than about 3.0, as expected (2.1, 1.9, and 2.5), whereas in the other three the ratios were much greater (5.7, 4.4, and 5.3).

Cysteine and mercapturate conjugates

The cysteine and mercapturic acid conjugates of paracetamol were detected only in the plasma of the patient with the most severe renal failure and the concentrations were low.

Plasma concentrations after stopping paracetamol

Paracetamol and its glucuronide and sulphate conjugates disappeared rapidly from the plasma in both volunteers and patients once paracetamol was discontinued (Table 2). Only the two patients with the most severe renal failure still had detectable amounts of retained conjugates in the plasma 4 days after stopping the paracetamol.

Discussion

Oral paracetamol is metabolized extensively by conjugation with glucuronic acid and sulphate. A small fraction of the dose is converted to a reactive intermediate metabolite, which is then conjugated with glutathione and excreted as cysteine and mercapturate conjugates. Over 90% of a therapeutic dose is normally excreted in the urine as metabolites within 24 h [Forrest et al. 1982]. In patients with renal failure the ability of the kidney to eliminate polar metabolites is limited, and during repeated dosing significant accumulation of paracetamol conjugates was expected.

When patients with chronic renal failure were given 1 g of paracetamol 3 times daily for 10 days, plasma concentrations of paracetamol were higher than in healthy volunteers taking the same dose. This confirms similar findings in a single-dose study of paracetamol disposition in patients with renal failure [Prescott et al. 1989]. The mechanisms are unknown, but it has been suggested that biliary excretion of paracetamol conjugates may become more important when their urinary excretion is reduced in renal failure [Siegers and Klaassen 1984]. In such circumstances the enterohepatic circulation of glucuronide and sulphate metabolites may be increased and paracetamol may be regenerated by hydrolysis of the conjugates by gastrointestinal flora, with subsequent reabsorption of the parent drug [Verbeeck et al. 1981].

The mean plasma concentrations of the sulphate and glucuronide conjugates were markedly higher in the patients with renal failure, and steady state was reached by about the 2nd and 6th days respectively. The plasma concentrations of the glucuronide conjugate at steady state depended on the severity of the renal failure, but this relationship did not hold for the sulphate conjugate. Similarly, the steady-state concentration of the glucuronide conjugate could be accurately predicted from single-dose studies, but the accumulation of the sulphate conjugate was much less than predicted in 3 of the 6 patients.

Long-term or high-dose paracetamol therapy is associated with a reduced rate of formation of the sulphate conjugate, which is due primarily to sulphate depletion [Levy & Yamada 1971; Lin & Levy 1981; Clements et al. 1984], although the serum concentrations of inorganic sulphate are not invariably lower than normal [Hendrix-Treacy et al. 1976]. This may have limited the amount of sulphate conjugates formed over the 10-day dosing period in some of our patients. However, chronic renal failure is often associated with sulphate retention [Freeman & Richards 1979], and so depletion is less likely to occur even with large doses of paracetamol [Lin & Levy 1982]. The patients were taking other medications and it is conceivable that this might have influenced paracetamol metabolism.

Whatever the mechanism, it is clear that the sulphate conjugation of paracetamol was limited in our patients. Consequently, the concentrations of the glucuronide conjugate were relatively higher, as shown by the increased ratio of glucuronide:sulphate conjugates at steady state. Normally the ratio of glucuronide:sulphate conjugates formed is about 2:1 [Forrest et al. 1982]. This relationship was maintained in the three patients in whom the predicted and observed steady-state concentration of sulphate conjugate were similar, but not in the other three. Further studies of the apparent non-linear sulphate conjugation of paracetamol in patients with renal failure are indicated, at a range of paracetamol doses, with simultaneous measurement of the serum inorganic sulphate concentrations.

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